RC
620
N272
1981
v.2

N I A D D K

ANNUAL REPORT

October 1, 1980 through September 30, 1981

D 50280 >>$0.39

and Kidney Diseases (U.S.)
NIADDK annual project report

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 34002-17 LMB

PERIOD COVERED

October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Enzyme Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. R. Davies	Chief, Sec. on Mol. Structure	LMB NIADDK
OTHER:	E. W. Silverton	Research Chemist	LMB NIADDK
	R. Bott	Staff Fellow	LMB NIADDK
	G. Gilliland	Staff Fellow	LMB NIADDK
	L. Sjolin	Visiting Fellow	LMB NIADDK

COOPERATING UNITS (if any)

Alexander Wlodawer, NBS

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.2	PROFESSIONAL: 3.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aspartic protease from Rhizopus chinensis has been analyzed as a complex with the inhibitor pepstatin. A mechanism of substrate binding and cleavage has been proposed. Crystals have been obtained of mouse renin.

Some preliminary data collection has begun with the enzyme S-adenosyl methionine synthetase.

The x-ray structure of bovine ribonuclease A has been refined to 2.0 A resolution. The neutron diffraction investigation of this enzyme has been completed at 2.8 A and is being extended to 2.0 A.

RC
62r
N272
1981
2

Project No. Z01 AM 34002-17 LMB

Project Description:

Objectives: The determination of the three-dimensional structures of enzymes with a view to understanding their mechanism of action.

Methods Employed: X-ray diffraction analysis of single crystals including heavy atom isomorphous replacement, model building, coordinate refinement in both real and reciprocal space, and computer graphics.

Major Findings: 1) The investigation of the crystal structure of the aspartic protease from Rhizopus chinensis has been continued. Data have been collected to 1.8 A with the intention of refining the structure to this resolution. The binding of the aspartic protease inhibitor pepstatin, a peptide obtained from streptomyces, has been analyzed as a complex with the enzyme. The role played by residue statine (4-amino-3-hydroxy-6-methyl heptanoic acid) at position 4 in the inhibitor has been established and it has been verified that statine approaches a tetrahedral intermediate. Based on the statine binding a model of the substrate on the enzyme surface has been constructed and from an examination of this a mechanism of action of the enzyme has been proposed.

2) The murine aspartic protease renin has been crystallized and some of the crystal properties have been determined. The crystals, although still small, diffract to ∿ 3 A resolution and appear to be suitable for a full three-dimensional analysis of the protein.

3) The enzyme bovine ribonuclease A has been studied by neutron diffraction in collaboration with Alexander Wlodawer at the National Bureau of Standards. In order to obtain a suitable starting point it was necessary to carry out an extensive refinement of the X-ray structure at 2.0 A resolution. This refinement is now complete and the neutron diffraction refinement is in progress and approaching completion.

Significance to bio-medical research and the program of the Institute: The acid protease is one of a large class of enzymes that include pepsin, renin, and the cathepsins. It has sequence homology with other members of the class and the elucidation of its structure should also reveal the mechanism of action of these proteins.

The application of neutron diffraction to biological structure introduces a new dimension in that this technique can locate the positions of protons and deuterons. This has profound significance for the understanding of the molecular mechanism of action of enzymes, and is being applied in this case to the digestive enzyme, ribonuclease.

Publications:

Cohen, G.H., Silverton, E.W. and Davies, D.R.: The refined crystal structure of γ-chymotrypsin at 1.9 A resolution: Comparison with other pancreatic serine proteases. J. Mol. Biol., in press.

Wlodawer, A. and Sjolin, L.: Orientation of histine residues in ribonuclease A: A neutron diffraction study. Proc. Natl. Acad. Sci. USA 78: 2853-2855, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 34003-13 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Crystal Structure Investigation of Proteins of the Immune System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. R. Davies	Chief, Sec. on Mol. Structure	LMB NIADDK
OTHER:	G. H. Cohen	Research Chemist	LMB NIADDK
	G. Gilliland	Staff Fellow	LMB NIADDK
	E. W. Silverton	Research Chemist	LMB NIADDK
	S. W. Suh	Visiting Fellow	LMB NIADDK

COOPERATING UNITS (if any)
J.J. Metzger, Immunology Branch, NCI; M. Klein, N. Haeffner-Cavaillon, D. Isenman, C. Rivat, M. Navia, K.J. Dorrington, University of Totonto

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.6	PROFESSIONAL: 3.6	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Further refinement has been carried out of McPC603 Fab, a murine phosphocholine specific myeloma protein.

An analysis of the structure of the murine galactan-specific J539Fab at 2.5 A has been commenced.

Project Description:

Objectives: A comparative investigation of the crystal structure of the Fab fragment of immunoglobulin molecules that bind strongly to several different antigens. In this way, it is hoped to map out the binding site of antibodies and to illustrate the mechanism of antigen-antibody interaction.

Methods Employed: Preparation and analysis of pure materials. Crystallization and x-ray diffraction analysis with: a) heavy atom isomorphous replacement, b) rotation function, c) molecular replacement in real space. Model building. Computer comparison of structures. Computer automated refinement of phases and atomic coordinates.

Major Findings: 1) The structure of McPC603 has been continued to be refined at 2.7 A resolution. Refinement has resulted in considerable improvement in the binding site residues. Analysis of the phosphocholine difference electron density maps show the phosphocholine binding site and demonstrate that only minor conformational changes occur upon binding hapten.

2) The x-ray oscillation camera data for J539, a mouse galactan - specific myeloma protein has been processed to give a complete 2.5 A data set. The previous 4.5 A data were used to obtain a convincing fit with a partially refined model, using coordinates from the McPC603 model. This model is now being applied to the 2.5 A data in order to obtain a refined model. This analysis is proceeding well and a high resolution structure of the first sugar-binding Fab can be expected soon.

3) Continued attempts have been made to obtain crystals of the porcine transplantation antigens, in collaboration with a group in the Transplantation Biology Section, Immunology Branch, NCI. The antigens have been purified by papain treatment of crude membrane preparations from spleen. The isolated protein, which probably contains two distinct gene products, has been used to screen for crystallization conditions to produce crystals suitable for x-ray diffraction studies. Presently two crystal forms have been produced, needles and very thin plates. Neither appear suitable at this stage for x-ray diffraction.

Publications:

Klein, M., Haeffner-Cavaillon, N., Isenman, D.E., Rivat, C., Navia, M.A., Davies, D.R., and Dorrington, K.J.: Expression of biological effector functions by immunoglobulin G molecules lacking the hinge region. Proc. Natl. Acad. Sci. USA 78: 524-528, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 35000-17 LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chemical and Structural Investigations of Nucleic Acids and Related Substances

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Todd Miles	Chief, Sec. on Org. Chem.	LMB	NIADDK
OTHERS:	Frank B. Howard	Research Scientist	LMB	NIADDK
	Kunal B. Roy	Visiting Associate	LMB	NIADDK

COOPERATING UNITS (if any)
Prof. Neville Kallenbach, University of Pennsylvania
Dr. Girjesh Govil, TIFR, Bombay, India

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Organic Chemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.3	PROFESSIONAL: 3.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has the long range objective of studying the chemistry and structure of nucleotides, polynucleotides and nucleic acids and the application of these studies to the understanding of biochemical and biological processes. Topics of current interest include: (1) structure and reactivity of polynucleotides and the relation between the two; (2) stabilization of ordered forms of nucleic acids and polynucleotides; (3) spectroscopic studies of polynucleotides: infrared, ultraviolet, and circular dichroism; (4) design and synthesis of new polynucleotides having properties required for the foregoing studies.

Project Description:

A Stereospecific Complex of Poly(I) with Ammonium Ion

We have previously discovered that poly(I) helix formation exhibits a striking dependence on the nature of the alkali metal ion present in solution and have attributed this effect to size-specific chelation of metal ions in the axial channel of the 4-stranded helix. We have now found that specific complex formation between poly(I) and NH_4^+ can be brought about by freezing and thawing the solution. Accounting for the properties of this stable complex, however, presents more difficult problems than do the alkali metal ion complexes. In particular, the NH_4^+ ion imposes major stereochemical constraints upon complexing which are not present in the spherically symmetrical alkali metal ions. All 4 protons must be hydrogen bonded, tetrahedral symmetry of the NH_4^+ ion must be satisfied in the complex, and acceptable hydrogen bond lengths and angles must be present in a structure which is consistent with the general helix geometry. We have proposed the following model for the complex. The NH_4^+ ions are located on the 4-fold axis midway (1.7Å) between the planar arrays of the bases. The distance from this site to each of the nearest 8 carbonyl oxygens is ~2.8Å, a favorable value for NH···O hydrogen bonds. The bonds are nonlinear by only 15°, well within the accepted range. Two of the protons are bonded to diagonally opposite carbonyl oxygens in the planar array above the binding site and two to those in the array below. The calculated HNH angle is then 108°, in good agreement with the tetrahedral value. The model is thus able to satisfy the geometrical and chemical constraints imposed by the NH_4^+ ion.

Binding Isotherms of Nucleosides and Polynucleotides by Continuous Ultrafiltration at Constant Volume

Equilibrium binding isotherms have been measured as a function of temperature for the interaction of 2- and 8-aminoadenosine with poly (U) by continuous, constant-volume ultrafiltration. An analytical method is described for determining the entire isotherm in a single experiment on a preequilibrated ligand-polymer solution. The method is shown to produce the same results as classical equilibrium dialysis but to be much more rapid and convenient. Values of the enthalpy, equilibrium constant, and free energy of stacking as a function of temperature are derived from the binding data. Stoichiometry of interaction is obtained from the diafiltration profiles as well as from the binding isotherms. For 2-aminoadenosine the association constant with poly (U) is 2083 M^{-1}, the enthalpy -21.4 kcal/mol, and the stacking free energy -2.0 kcal/mole at 8° in 0.15M Na^+ at pH 7. For 8-aminoadenosine under the same conditions the corresponding values are 3773 M^{-1} -19.9 kcal/mole, and -2.2 kcal/mole.

Spectroscopic Detection of Premelting Open States in Polynucleotide Helices

Professors Neville Kallenbach and Walter Englander and their colleagues had previously found evidence from isotope exchange experiments (tritium and deuterium exchange) suggesting that nucleic acid helices have significant (e.g. 1-5%) open or unpaired regions at temperatures well below the melting region. As part of a collaborative project with them we have examined the problem by infrared spectroscopy, which was shown by our previous work to be very sensitive to base pairing. Computerized infrared difference spectroscopy has demonstrated the existence of unpaired states at low temperatures and given estimates of the extent of these open regions in qualitative agreement with the isotope exchange experiments. We have also carried out CD measurements on the Jasco 500, again using computerized difference spectra to detect open states at low temperature. These results are also in general agreement with the others, though there are quantitative differences among the three methods.

Ordered Structures of Polyxanthylic Acid

Our previous work has indicated that poly(X) in neutral solution (ionized at N3) forms a 4-stranded helix with size-specific complexing of alkali metal ions in the axial channel and non-specific association of metal ions at the C2 enolate anion. Screening of interstrand repulsion of the negatively charged bases by the internal counterions is essential for helix stability. The acid form of poly(X) (unionized), however, has an entirely different structure for which we have proposed a 6-stranded helix. Further studies with other ions are consistent with these ideas. Tetraethylammonium ion is too large to fit in the axial channel of a 4-stranded helix and we find that no ordered poly(X) structure is formed at neutral pH in the presence of this ion. The acid structure, which should not depend on a central metal ion, is fully formed and highly stable in the presence of $^{+}NEt_4$. In an attempt to fit the larger axial channel of the proposed 6-stranded acid helix we have employed guanidinium ion. This planar ion has 6 hydrogen atoms suitably disposed to form hydrogen bonds to 6 xanthine carbonyl groups. We find that this ion does bind specifically to the acid structure, with some change of spectroscopic properties. With the 4-stranded neutral helix, however, no binding of guanidinium ion is detected.

Publications:

Mathelier, H.D., Howard, F.B., and Miles, H.T.: Circular Dichroism of Helices Formed by Purine Monomers with Pyrimidine Polynucleotides. Biopolymers 18:709-722, 1979.

Roy, K.B., Frazier, J. and Miles, H.T.: Polyxanthylic Acid: Structure of the ordered Forms. Biopolymers 18:3077-3087, 1979.

Muraoka, M., Miles, H.T., and Howard, F.B.: Copolymers of Adenylic and 2-aminoadenylic Acids. Effect of Progressive Changes in Hydrogen Bonding and Stacking on Interaction with Poly(uridylic acid). Biochemistry 19:2429-2439, 1980.

Howard, F.B., Roy, K.B., Frazier, J. and Miles, H.T.: Does Perchlorate Ion Change A•U Combining Ratios? Biopolymers 20:1089-1102, 1981.

Govil, G., Fisk, C.L., Howard, F.B., and Miles, H.T.: Spectroscopic Studies on the Structure of Poly(8-bromoadenylic acid): Effect of Glycosidic Torsion Angle on the Conformation and Flexibility in Polyribonucleotides. Biopolymers 20:573-603, 1981.

Miles, H.T.: Infrared Spectroscopy of Polynucleotides in D_2O and H_2O Solution. In Srinivasan, R. (Ed.): Biomolecular Structure, Conformation, Function and Evolution, Vol. 2, Physico-Chemical and Theoretical Studies. Oxford and New York, Pergamon Press, 1980, pp. 251-265.

Howard, F.B. and Miles, H.T.: A Stereospecific Complex of Poly(I) with Ammonium Ion. Biopolymers 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 35050-10-LMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Replication, Recombination and Repair of Microbial DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Jun-ichi Tomizawa	Chief, Sec. on Mol. Genetics	LMB	NIADDK
OTHER :	Tateo Itoh	Visiting Scientist	LMB	NIADDK
	Gerald Selzer	Staff Fellow	LMB	NIADDK
	Tapan Som	Visiting Associate	LMB	NIADDK
	Hisao Masukata	Visiting Fellow	LMB	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Genetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland

TOTAL MANYEARS: 5.5	PROFESSIONAL: 5.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transcription of plasmid ColE1 DNA by RNA polymerase in vitro starts at two sites in a region required for maintenance of the plasmid. Certain transcripts that start at one of the sites can be cleaved by RNase H and then act as primers for DNA replication. Transcription from the other site produces a RNA ∿ 108 nucleotides long (RNA I).

If purified RNA I is added to the transcription reaction containing RNase H, formation of primer is inhibited. Each RNA I can inhibit primer formation by the plasmid that specifies it but has no effect on primer formation by heterologous templates. Thus, the inhibition of primer formation by RNA I is incompatibility specific. Because RNA I does not inhibit initiation or propagation of transcription or the processing of preformed precursors, the step that is sensitive to inhibition is probably formation of the hybrid between the primer precursor and the template. This hybrid is the required substrate for RNase H.

PHS-6040
(Rev. 2-81)

Project Description:

Closed circular DNA of the plasmid ColEl of Escherichia coli can be replaced in vitro by a soluble enzyme system. No plasmid encoded protein is required for this replication. Initiation of replication can be demonstrated with a mixture of three highly purified E. coli enzymes: RNA polymerase, DNA polymerase I and ribonuclease H (RNase H). Transcription of ColEl DNA by RNA polymerase in vitro starts at two sites in a region required for maintenance of the plasmid. Certain transcripts that start at one of the sites can be cleaved by RNase H and then act as primers for DNA replication. Transcription from the other site produces a RNA ~ 108 nucleotides long (species I or RNA I). Transcripts analogous to the primer and RNA I of ColEl are produced when p15A or small derivatives of two other ColEl-compatible plasmids, CloDF13 and RSF1030, are used as template. If purified RNA I is added to the transcription reaction containing RNA H, formation of primer is inhibited. Each RNA I can inhibit primer formation by the plasmid that specifies it but has no affect on primer formation by heterologous templates. Thus, the inhibition of primer formation by RNA I is incompatibility specific. Because RNA I does not inhibit initiation or propagation of transcription or the processing of preformed precursors, the step that is sensitive to inhibition is probably formation of the hybrid between the primer precursor and the template. This hybrid is the required substrate for RNase H. To study the mechanism of inhibition of primer formation by RNA I, mutants of ColEl that have altered incompatibility properties have been isolated. Each mutant has a single base change at or near the center of at least one of the three palindromes in the region that specifies both the primer RNA and RNA I. Data from in vitro transcription studies show that the single base changes affect both the ability of RNA I to inhibit primer formation and, as well, the sensitivity of primer formation to inhibition by RNA I. RNA I hybridizes to transcripts originating at the primer promoter and the rate of hybridization is reduced by the single base changes. The in vivo properties of the mutant plasmids indicate that incompatibility between two plasmids can be attributed to inhibition of primer formation of at least one of the plasmids by the RNA I of the other.

Publications:

Tomizawa, J., Itoh, T., Selzer, G. and Som, T.: Inhibition of ColEl RNA primer formation by a plasmid-specifies small RNA. Proc. Natl. Acad. Sci. U.S.A., 78 1421-1425, (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36002-07 LMB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Novel Recombination Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Rosner	Research Biologist	LMB NIADDK
OTHER:	Atin R. Datta	Visiting Fellow	LMB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Microbial Genetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Environmental and genetic factors which influence transposition of Tn9 in E. coli have been studied. Acetate ion and several lipid solvents (Brij 58, NP40 and dimethylsulfoxide) increase the frequencies of Tn9, Tn5 and Tn10 transpositions. The C16 fatty acid, palmitic acid, reverses the effect of Brij 58. Compared to wild-type, a rho mutant (defective in transcription termination) shows a 20 fold lower frequency of transposition for Tn9 but is normal for Tn5 and Tn10. Strains deleted for the gal-attλ region of the chromosome are defective in a special transposition event wherein an IS1 or IS5, trnaspose to a distant chromosomal site (bgl).

Project Description:

Objectives: An understanding in molecular terms of transposition, a process involving recombination between certain discrete DNA sequences (transposons) and non-homologous target chromosomes. Particular attention has been paid to the regulation of IS*1*-mediated transposition.

Major Findings: (1) Previously, we reported that transposition of Tn*9* is enhanced 3-10 fold by 50 mM acetate. We now find that conversion of acetate to acetyl coA is necessary for this stimulation since no effect of acetate is found in mutants defective in the conversion of acetate to acetyl phosphate (*ack*$^-$) or of acetyl phosphate to acetyl coA (*pta*$^-$).

(2) We have also found that several lipid solvents (Brij 58, NP40 and dimethylsulfoxide) enhance transposition of Tn*9*. Surprisingly, the C16 fatty acid, palmitic acid, reverses the enhancing effect of Brij 58. These results suggest an involvement of lipid (membrane?) in transposition.

(3) A mutant of *E. coli*, *rho*ts15, which is defective in transcription termination shows a 20 fold lower than normal frequency of IS*1*, IS*5* and Tn*9* transposition. Transposition of Tn*5* and Tn*10* and lysogenization by Mu, however, are normal.

(4) The salicin-papillation assay of Wright has been used to identify a new gene in *E. coli* (tentatively called *sap*) which is involved in transposition. This gene is located between *gal* and *att*λ. More precise mapping is underway.

Significance to biomedical research: The discovery of transposable elements has led to a new understanding of how chromosomal rearrangements can occur. Transposition plays an important role in evolution, gene regulation and perhaps development. Transposition of drug resistance elements is of great importance in the epidemiology of infectious diseases and chemotherapy.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36051-13 LMB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Genetics and structure of the oncogenic virus, SV40

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert G. Martin, Chief, Section on Microbial Genetics, LMB		NIADDK
OTHER:	Ana Chepelinsky, Visiting Scientist	LMB	NIADDK
	Maria Hadjopoulos, Visiting Fellow	LMB	NIADDK
	Shaw S. Wang, Bio Lab Technician	LMB	NIADDK

COOPERATING UNITS (if any)

S. Wickner	Research Chemist	CLMB
A. Oppenheim	Visiting Scientst	CLMB

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Microbial Genetics

INSTITUTE AND LOCATION
NIADDK NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Analysis of the physical chemistry of linear DNA containing replication loops has demonstrated that the loops are remarkably unstable and are extruded as double stranded DNA at a significant rate even at temperatures 50° and more below the T_m. The rate limiting step in this extrusion is the rate of end melt and/or nucleation of the nascent strands, not the rate of branch migration. This unusual feature provides a general method for the isolation of origins of DNA replication.

In other experiments we have demonstrated that SV40 is not a potent mutagen and that its weak mutagenic activity is probably unrelated to its ability to transform.

PHS-6040
(Rev. 2-81)

Project Description:

Objective: To determine the molecular basis of transformation by SV40 and to determine how SV40 alters the normal pattern of DNA synthesis.

Major findings: (a) Both upon acute infection of nonpermissive cells by SV40 and in SV40 transformed cells the mutation rate has been reported to be significantly increased. This has led to a theory that SV40 transformation is analogous to chemical carcinogenesis. We have undertaken extensive analyses with temperature sensitive and deletion mutants of SV40 in both hamster and rat cells and can find no consistent increase in the mutation rate. (Hadjopoulos-Zannis and Martin)

(b) A number of revertants of temperature-insensitive cell lines generated by temperature sensitive viral mutants have been analyzed. All have lost expression of the SV40 tumor antigen. Analysis of the integrated SV40 sequences is now in progress. (Chepelinsky and Martin)

(c) A cell free system for the synthesis of DNA has been developed. Unfortunately, multiple attempts to demonstrate that this system can initiate DNA synthesis have been unsuccessful. (Chepelinsky, Martin in collaboration with A. Oppenheim and S. Wickner)

(d) We have demonstrated that linear double stranded DNA containing a replication loop is remarkably unstable even at temperatures 50° and more below the T_m for DNA. Our data suggest that end melt of the nascent strands (the short strands in the replication loop) is the rate limiting step. Following end melt of one of the strands, the second is extruded and the two ends of the partially melted short strands can now hybridize forming a Halliday structure. This structure then undergoes a random walk by branch migration eventually leading to extrusion of the nascent strands. The process only occurs in linear DNA because free rotation is necessary to permit branch migration to proceed. This bit of physical chemistry allows one to isolate origins of DNA replication at will. We are currently applying this technique to a number of different organisms. (Hadjopoulos and Martin)

Publications:

Martin, R. G. The transformation of cell growth and transmogrification of DNA synthesis by SV40. Adv. Cancer Res., 34: 1-68, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36101-07 LMB

PERIOD COVERED
October 1, 1980 - September 31, 1981

TITLE OF PROJECT (80 characters or less)
Energy conversion in biology, especially muscle contraction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terrell L. Hill	Chief Section on Theoretical Molecular Biology. LMB, NIADDK
OTHER:	Yi-der Chen	Research Chemist, LMB, NIADDK
	Takashi Tsuchiya	Visiting Associate, LMB, NIADDK

COOPERATING UNITS (if any)

Evan Eisenberg	Heart, LB
Alex Levitzki	Cancer, LMB

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Theoretical Molecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three different topics have been studied under this project: (a) we have completed an extensive four-year study of the general properties of cooperative steady-state enzymatic systems. Three-state Ising systems were studied further, as well as half-of-the-sites reactivity in a dimer; (b) double-stranded DNA can be unwound by ATP-dependent helicases and by treadmilling binding proteins, using the net free energy of DNA synthesis on the leading strand. These processes have been analyzed from a bioenergetic point of view, and a possible helicase model has been proposed; (c) we are continuing our work on explicit kinetic models of muscle contraction.

PHS-6040
(Rev. 2-81)

Three different topics have been studied under this project:

(a) (Hill, Chen, Tsuchiya, Levitzki). We have completed an extensive four-year study of the general properties of cooperative steady-state systems. Three-state Ising systems were studied further, as well as half-of-the-sites reactivity in a dimer. The particular application to the cooperative regulated-actin myosin ATPase will be taken up again, in the near future, using the Monte Carlo method.

(b) (Hill, Tsuchiya). Double-stranded DNA can be unwound by ATP-dependent helicases and by treadmilling binding proteins, using the net free energy of DNA synthesis on the leading strand. These processes have been analyzed from a bioenergetic point of view, and a possible helicase model has been proposed.

(c) (Eisenberg, Hill, Chen). We are continuing our work on explicit kinetic models of muscle contraction. Calculations are in progress on an 8-state, single-site model with biochemistry corresponding to new results of Eisenberg's group. Related to this, we have made a detailed study of the extent to which free energy transfer can be followed step-by-step through a transducing biochemical cycle, as in membrane or muscle systems.

Publications

Hill, T. L. and Chen, Y.: Three-state steady-state Ising systems: Monte Carlo and Bragg-Williams treatments. Proc. Natl. Acad. Sci. USA 78: 4-8 (1981).

Tsuchiya, T.: Oscillating properties of interacting three-state cyclic enzyme systems. J. Chem. Phys. 73: 3791-3796 (1980).

Hill, T. L. and Levitzki, A.: Subunit nieghbor interactions in enzyme kinetics: half-of-the-sites reactivity in a dimer. Proc. Natl. Acad. Sci. USA 77: 5741-5745 (1980).

Hill, T. L. and Tsuchiya, T.: Theoretical aspects of translocation on DNA: ATPases and treadmilling binding proteins. Proc. Natl. Acad. Sci. USA, in press.

Hill, T.L. and Eisenberg, E.: Can free energy transduction be located at some crucial part of the enzymatic cycle? Q. Revs. Biophysics, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36102-10 LMB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Statistical thermodynamics of protein and polynucleotide systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terrell L. Hill	Chief, Section on Theoretical Molecular Biology, LMB, NIADDK	
OTHER:	Yi-der Chen	Research Chemist	LMB, NIADDK
	Takashi Tsuchiya	Visiting Associate	LMB, NIADDK

COOPERATING UNITS (if any)

Evan Eisenberg	Heart, LB
Lois Greene	Heart, LB

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Theoretical Molecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Four separate topics have been studied under this project: (a) The head-to-tail treadmilling of subunits in actin filaments and in microtubules has been studied further. Our earlier bioenergetic analysis has been extended to take care of a three-state NTPase cycle, which is important under some experimental conditions; (b) further work has been done on the theory of binding of myosin to regulated actin; (c) as a first step in studying the structure of covalently-closed circular DNA molecules in solution, we have devised an efficient method of generating ring polymers of the freely jointed model; and (d) the structure of circular DNA molecules is being investigated using ring polymers of the freely jointed model by adding bending and twisting energies to the ring.

PHS-6040
(Rev. 2-81)

Four different topics have been studied under this project:

(a) (Hill) The head-to-tail treadmilling of subunits in actin filaments and in microtubules has been studied further. Our earlier bioenergetic analysis has been extended to take care of a three-state NTPase cycle, which is important under some experimental conditions. The steady-state polymer length distribution has been derived; it has the same mathematical form as at equilibrium but the parameters are different. Assembly or disassembly of subunits under a mechanical force has been included in the theory — this is important for changes in shape of cells (actin) or for the movement of chromosomes in mitosis (microtubules).

(b) (Hill, Tsuchiya, Eisenberg, Greene) Further work has been done on the theory of binding of myosin to regulated actin (actin + tropomyosin + troponin + Ca^{++}). Heavy meromyosin attaches to two adjacent actin binding sites. The statistical mechanics of this binding, taking into account the seven-site units of regulated actin, has been worked out by an exact matrix theory. Corresponding experiments are in progress by Eisenberg and Greene at N.I.H. In other work, an alternative model for one-headed S-1 binding on regulated actin has been studied. In this model, the first S-1 in a unit of seven sites binds with difficulty but further S-1 molecules bind relatively easily, owing to a more favorable position of tropomyosin imposed by the binding of the first S-1.

(c) (Chen) As a first step in studying the structure of covalently-closed circular DNA molecules in solution, we have devised an efficient method of generating ring polymers of the freely jointed model. The method is several orders faster than the usual (direct) Monte Carlo method and thus makes it possible the first time to study the statistics of large ring polymers of this model.

(d) (Chen) Many biological processes in circular DNA molecules, such as replication, transcription, recombination, etc., are closely related to the tertiary structure of the DNA molecule. For example, these processes are greatly enhanced by the presence of negative superhelices. In gyrase action, the directional orientation of the two crossing segments determines the increase or decrease of the linking number. In this study, the structure of circular DNA molecules is being investigated using the ring polymers of the freely jointed model by bending and twisting energies to the ring. Specifically, the radius of gyration, the writhing number, and the orientational distribution function of two distant segments of circular DNA molecules will be calculated as a function of the linking number and the persistence length of the DNA.

Publications

Hill, T. L.: Steady-state head-to-tail polymerization of actin or microtubules II. Two-state and three-state kinetic cycles. Biophys. J. 33: 353-372 (1981).

Hill, T. L.: Microfilament or microtubule assembly or disassembly against a force. Proc. Natl. Acad. Sci. USA, in press.

Tsuchiya, T. and Hill, T.L.: n-Mer binding on a one-dimensional lattice of two-state m-site cells: heavy meromyosin on regulated actin as an example. Biopolymers, in press.

Hill, T. L.: Binding under a molecular umbrella as a cooperative statistical mechanical system: tropomyosin-actin-myosin as an example. Biophys. Chem., in press.

Chen, Y.: Monte Carlo study of freely jointed ring polymers. I. Generation of ring polymers by dimerization method. J. Chem. Phys. 74: 2034-2038 (1981).

Chen, Y.: Monte Carlo study of freely jointed ring polymers. II. The writhing number. J. Chem. Phys., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36103-10 LMB

PERIOD COVERED

October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Ion transport across the nerve axon membrane

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yi-der Chen	Research Chemist	LMB	NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Theoretical Molecular Biology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

No new developments on this project at this time.

PHS-6040

Annual Report of the
Mathematical Research Branch
National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases

The development of a mathematical model to describe the transport of oxygen in solutions of hemoglobin and myoglobin and a numerical method for its solution have been reported previously. The chemical kinetics are described by the single step reaction $P + X \rightleftharpoons PX$. The method is extended to take into account the cooperative properties induced by the four step reaction (Adair) of the hemoglobin-oxygen chemical kinetics. The magnitude of the facilitated transport of oxygen for the model that includes the cooperative properties of hemoglobin is compared with the one for the model associated with the single step reaction. Some features contained in the former that are not available in the single step model are shown to favor the facilitated transport. The results for hemoglobin as it occurs in the red cells make it plausible that facilitation may contribute to the transport of oxygen in the red cell inside the systemic capillaries. A manuscript "Transport of Oxygen in Solutions of Hemoglobin and Myoglobin" to be published in *Mathematical Biosciences* is now in press.

All of the published theoretical studies on facilitated transport have assumed homogeneous spatial properties. In the striated muscle there exist membranes that may impede the diffusion of the carrier myoglobin. A theoretical study is undertaken to analyze the transport in the presence of membranes on the diffusion path. The numerical method above has been extended to include the nonhomogeneous spatial parameters as they occur in the striated muscle. For the numerical computations the values of the parameters were chosen to comprise those of physiological relevance. The numerical results indicate that the presence of membranes tends to decrease the facilitation. For the nonlinear chemical kinetics of oxygen with the carrier, this decrement also depends on the location of the membrane. At the higher oxygen concentration side of the membrane the flow of combined oxygen is transferred to the flow of dissolved oxygen. The reverse process occurs at the lower concentration side. Jump discontinuities on the concentration of the oxygen-carrier compound at the membranes are associated with these transfers. Let Δ^i be absosolute value of the jump in the concentration at the i-th membrane. It was shown that the facilitated transport can be represented by

$$F_f = \frac{D_2}{\ell}\left[x_2^{eq}(x_1^0) - x_2^{eq}(x_1^\ell) - \{\Delta_0^+ + \sum_{i=1}^{n}(\Delta_i^- + \Delta_i^+) + \Delta_{n+1}^-\}\right],$$

where D_2 is the diffusion coefficient of the oxygen-carrier, ℓ is the width of the slab, + represent the higher oxygen concentration side of the membrane, - represent the lower concentration side, n is the number of membranes and $x_2^{eq}(x_1^0)$, $x_2^{eq}(x_1^\ell)$ are the equilibrium concentrations of oxygen-carrier at the faces of the slab. One observes from this expression that the decrement in facilitation depends on the cummulative effect of the jumps. Let σ_i denote the boundary layer width at the i-th membrane. When the intermembrane distance is larger than $2\sigma_i$, it was shown that a relationship exists between Δ^i, the total flow of oxygen and the slope of the oxygen dissociation curve. These relations define an algebraic system whose solution gives the facilitated flow. This algebraic system enables one to find the facilitated flow directly without obtaining the profiles of concentration of the substrates. (Jose M. Gonzalez-Fernandez and Susie E. Atta).

We have previously carried out theoretical calculations of responses in dendritic neurons to various spatial distributions of synaptic input and to injection of current at different dendritic locations. Many of these earlier computations took advantage of idealized branching geometry (e.g. Rall, 1960; Rall and Rinzel, 1973, 1974). Other computations (Rall, 1964, 1967) introduced compartmental models which permit specified departures from idealized branching and from uniform membrane properties; this makes it possible for computations to be matched as closely as desired to actual experimental situations. We have now begun a program of matching such computations to several different experimental preparations in consultation and collaboration with several different colleagues. The neurons to be studied in detail are (1) motorneurons from the lobster stomatogastric ganglion (anatomical and electrophysiological data already obtained by Dr. John Miller at UCSD; now a guest worker here), (2) mitral and granule cells of turtle olfactory bulb (data already obtained by Prof. Gordon Shepherd and colleagues at Yale Univ. Med. Sch.) and (3) motorneurons of cat spinal cord (data obtained by Dr. Robert Burke and colleagues in Lab. of Neural Control, NINCDS). The computations make use of a large electrical network modeling program, "SPICE" which runs on the IBM 370. By adapting our mathematical model to this program, we can specify any combination of electrical membrane properties and any neuron geometry. We have begun to investigate the attenuation and phase shift of sinusoidal current inputs to the soma of a model neuron. The results show the same trends as those observed experimentally in motorneurons of the lobster stomatogastric ganglion. We are currently investigating the effects of including more or less detail in the model to represent the known details of the dendritic branching of these lobster neurons. Consultations have been carried out both with Prof. Gordon Shepherd and with Dr. Robert Burke. The overall objective of these studies is to gain and test specific insights relevant to the integrative properties of dendritic neurons which receive inputs from many afferent sources. (Wilfrid Rall and John Miller).

Arbitrary stationary homogeneous media generically behave in one of four canonical ways near a uniform equilibrium point. Multiple scales, bifurcation, and symmetry methods can be used to derive canonical equations governing the behavior of the full system. These equations have been derived and analyzed. Three papers are in preparation.

An improved set of bifurcation equations for hexagonal lattices is applied to a problem of visual illusions. Hysteresis, multiple patterns, and onset of illusions under periodic stimuli is predicted. Phenomena ranging from drug induced hallucinations to deep pressure and flicker phosphenes can be easily explained by this theory.

Neuronal networks which have strong self excitation can display a variety of rotating and standing oscillatory waves. The form of these waves, their frequency, and dispersive qualities resemble physiologically recorded epileptic waves in rabbit cortex. A manuscript describing these waves is in preparation.

Multiple scaling is used to elucidate the behavior of two coupled oscillators with nearly rationally related frequencies. The ranges of n:m phase locking are described and applied to various biological and physical problems. The results are to appear in J. Math. Biology, 1981.

A chain of coupled oscillators which models the rhythmic properties of the small intestine is analyzed. A geometric method is used to explain the onset of "frequency plateaus", a well known but poorly understood property of the intestine. A simplified "phase" model is also used which qualitatively agrees with the more complex system.

A "degenerate" homoclinic orbit is periodically perturbed. This problem is analogous to stimulating a space clamped nerve axon or any similar medium. Regimes of phaselocking are found which correspond to periodic solutions to the full problem. Singular perturbation methods reduce the system to a canonical Hill's equation:

$$u_{tt} = -uQ(t) \; ; \quad Q(t + T) = Q(t).$$

A manuscript is in preparation. (G. B. Ermentrout and J. Rinzel).

We have analyzed oscillatory solutions to a modified Field-Noyes model for the Belousov-Zhabotinskii chemical reaction. Bursting patterns of oscillation have been observed experimentally in a continuous flow, stirred tank reactor. By considering a range of parameter values and simplified models, we explain three qualitatively different bursting phenomena. In one case, bursting is due to a hysteresis mechanism in which a dynamic variable slowly sweeps back and forth through values for which the (non-flow) batch reactor model has both a stable steady state and a stable limit cycle. Our results have been presented at several conferences; a manuscript is in final preparation. (J. Rinzel and W. C. Troy).

We previously observed, for a bistable FitzHugh-Nagumo reaction-diffusion model, that certain transition-type traveling waves may be reflected by local disturbances and others may not. In a idealized formulation, we analyze the reflection problem by treating the local disturbance as a specified boundary condition. In one case, we prove that a stable stationary solution exists; we predict and observe numerically that a wave will approach this stationary solution and will not be reflected. We conclude that reflection occurs for cases where a stationary solution is either unstable or does not exist. A manuscript, with D. Terman, has been submitted to the SIAM Journal of Applied Mathematics. (J. Rinzel).

Various aspects of mathematical modeling in cellular neurobiology were reviewed and presented by invitation at two conferences: Mathematical Aspects of Physiology (Salt Lake City, Utah) and NATO Advanced Study Institute, Nonlinear Phenomena in Physics and Biology (Banff, Canada). A paper will be published in proceedings for each conference. (J. Rinzel).

A simple kinematic approximation for the x-t trajectories, and speeds, of propagating nerve impulses has been proposed (R. N. Miller and J. Rinzel, Biophys. J. 34 (1981) 227-259). By using the Hodgkin-Huxley model as a test case for a two-pulse simulation, we are comparing this procedure with other approximate descriptions in the literature. (J. Rinzel and A. C. Scott).

We have studied dynamic behavior in a simple, one-variable, reaction-diffusion model for the cases of either excitable or oscillatory reaction kinetics (G. B. Ermentrout and J. Rinzel, J. Math. Biology 11 (1981) 269-294)

We are now investigating how solutions of the simple phase model are useful for asymptotically solving certain, more complicated, models. Spatially uniform oscillations are obtained by a singular perturbation procedure for a parameter near its critical value (the infinite period case). (J. Rinzel and G. B. Ermentrout).

All previously reported results regarding mean residence times, linear output of stationary systems and lag-times in presteady-state enzyme systems have been written up for publication and are in press. The practical and applicable aspects of the lag-time studies for coupled enzyme systems have been greatly extended to specify which rate parameters can be estimated from the lag-time alone or from lag-time in conjunction with steady-state studies of the usual type. Conditions which shorten the lag-time have been prescribed and are of some importance in biochemical assay with coupled enzyme systems. (John Z. Hearon).

The need for validation in kidney modelling, especially of solute and water balance, continues to be evident in the leterature. A six-tube vasa recta model (R. Mejia, J. L. Stephenson and R. J. LeVeque, A Test Problem for Kidney Models, Math. Biosci. 50:129-131, 1980) has been published for this purpose.

The existence of multiple steady states for certain models of the renal medulla has been recognized by us for some time. Methods for locating and classifying these states have been devised (R. Mejia, A Path Following Method for Obtaining a Connected Component of the Solution Surface of a Convection-Diffusion Model in Fluid Flow, abstract, Annual Fall Meeting of the Maryland-District of Columbia-Virginia section of the Mathematical Association of America, November 1980). A method for obtaining such solutions for a multi-nephron kidney model and for studying the transition from one state to another as a function of renal parameters has been described (R. Mejia and J. L. Stephenson, Multiple Solutions of Convection-Diffusion Equations Describing the Renal Concentrating Mechanism, Numerical Methods for Engineering, GAMNI 2, 2nd International Congress, 1003-1013, 1980). The main conclusions of these investigations are (1) multiple stable steady states appear to be physiologically plausible; (2) such states are connected by hysteresis loops that might stabilize operation in substantially different regimes; (3) some paths, e.g., as a function of certain model parameters, are forbidden, which suggests appropriate experiments.

An interactive continuation algorithm called SCOUT due to H-O. Peitgen, H. Jürgens and D. Saupe at Universität Bremen, has been implemented on the PDP-10 and PDP-11 computers at the NIH Computer Center. It consists of a set of simplicial continuation utilities that accept a simple, FORTRAN, description of the set of equations whose roots are sought. It will homotope to a root from an initial guess; it will continue as a function of a parameter; allows for a variable mesh size; and uses a perturbation technique to resolve bifurcation points or seek disjoint solution branches. SCOUT has been used to map the solution surface for some analytic models and has served to verify results obtained for some small (up to 15 finite difference equations) discretized models using methodology described below.

SCOUT, although at the forefront of the state-of-the-art in numerical continuation, cannot begin to handle the nonlinear systems with hundreds of unknowns that arise in multinephron kidney models. Hence, the (partitioned) algorithm used to solve the time dependent kidney equations for many nephrons (R. Mejia and J. L. Stephenson, Numerical Solutions of Multinephron Kidney Equations, J. Comput. Phys. Vol. 32, Number 2, 235-246, 1979) has been extended. An adaptive multigrid method for solution of a multipoint boundary value problem has been developed. The $O(h^2)$ approximation used to solve the tubular equations in the partitioned algorithm has been replaced by an $O(h^4)$ scheme (R. P. Tewarson, On the Use of Simpson's Rule in Renal Models, Math. Biosci., to appear). These have been solved symbolically (R. Mejia and J. L. Stephenson, Symbolics and Numerics of a Multinephron Kidney Model, 1979 MACSYMA User's Conference, Washington, D. C., 596-603. The interstitial variables and the boundary equations are discretized using a difference scheme with space mesh size of h/2, which is conservative. The partitioned system is then solved using an adaptive, alternating method (R. Mejia and J. L. Stephenson, An Alternating Method of Solution of a Multipoint Boundary Value Problem, SIAM 1979 Fall Meeting, Denver, Colorado). Tests indicate that the increased accuracy in conjunction with a coarser mesh will permit solution of models with several nephron populations, something that is required, for example, in the study of multisolute systems.

Work has continued on approximate analytic solution of central core models of the renal medulla. Earlier analytic work has been extended by deriving general mass balance equations for an n-stage single solute central core model of the renal medulla. When these equations are combined with a previously derived approximate relation between axial volume flow, concentration, solute permeability, hydraulic permeability, and reflection coefficient; we are led to implicit equations for the concentration at the various medullary levels. For selected cases these can be solved to give a closed analytic solution, but in general they must be solved numerically. To do this and to explore the parameter space of these models, we have used the continuation algorithm called SCOUT described above. The results of these studies are now being written up for publication. (John Stephenson, R. Mejia and B. Kellogg).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,000-08 MRB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mathematics of kinetics and reaction-transport systems.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Z. Hearon	Chief, MRB	MRB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project continued to focus upon applications of linear algebra, matrix and vector space methods to problems in biology. Applications were heavily involved in compartmental analysis; linear control theory, and generalized kinetics. No small part of the project was devoted to meeting purely mathematical problems which arose on their proper abstract grounds.

PHS-6040
(Rev. 2-81)

All previously reported results regarding mean residence times, linear output of stationary systems and lag-times in presteady-state enzyme systems have been written up for publication and are in press. The practical and applicable aspects of the lag-time studies for coupled enzyme systems have been greatly extended to specify which rate parameters can be estimated from the lag-time alone or from lag-time in conjunction with steady-state studies of the usual type. Conditions which shorten the lag-time have been prescribed and are of some importance in biochemical assay with coupled enzyme systems. The project is hereby terminated.

Publications:

Hearon, J. Z.: Residence times in compartmental systems with and without inputs. Math. Biosci. (in press).

Hearon, J. Z.: Asymptotic output of compartmental systems. Math. Biosci. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,001-08 MRB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mathematical formulations and analysis relevant to experimental neurophysiology.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Wilfrid Rall	Senior Research Physicist	MRB, NIADDK
OTHER:	John Miller	Guest Worker (Postdoc. Fellow)	MRB, NIADDK

COOPERATING UNITS (if any)

Prof. Gordon Shepherd Yale Univ. Med. School New Haven, CT	Dr. Robert Burke LNC, NINCDS, NIH

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.7	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

For several years, this project has been concerned with constructing, developing and testing a group of interrelated mathematical models, relevant to experimental neurophysiology and neuroanatomy. Together these models provide a theory that can account for various sequences of events in the soma and dendritic branches of a single neuron, and for field potentials generated by certain cortical populations of neurons. Computational experiments performed with these models provide theoretical predictions that have been compared with experimental results obtained by colleagues with motoneurons of cat spinal cord, and with the mitral cell and granule cell populations of rabbit olfactory bulb. Resulting interpretations contribute to understanding of dendritic synaptic input and of dendro-dendritic synaptic interactions. These results have appeared in the Biophysical Journal and in the Journal of Neurophysiology. A comprehensive chapter, entitled "Core conductor theory and cable properties of neurons" (in The Handbook of Physiology: The Nervous System, Vol. I, Cellular Biology of Neurons, American Physiological Society 1977) summarizes some of these results. Detailed application is now being made to motor neurons of lobster stomatogastric ganglion.

We have previously carried out theoretical calculations of responses in dendritic neurons to various spatial distributions of synaptic input and to injection of current at different dendritic locations. Many of these earlier computations took advantage of idealized branching geometry (e.g. Rall, 1960; Rall and Rinzel, 1973, 1974). Other computations (Rall, 1964, 1967) introduced compartmental models which permit specified departures from idealized branching and from uniform membrane properties; this makes it possible for computations to be matched as closely as desired to actual experimental situations. We have now begun a program of matching such computations to several different experimental preparations in consultation and collaboration with several different colleagues. The neurons to be studied in detail are (1) motorneurons from the lobster stomatogastric ganglion (anatomical and electrophysiological data already obtained by Dr. John Miller at UCSD; now a guest worker here), (2) mitral and granule cells of turtle olfactory bulb (data already obtained by Prof. Gordon Shepherd and colleagues at Yale Univ. Med. Sch.) and (3) motorneurons of cat spinal cord (data obtained by Dr. Robert Burke and colleagues in Lab. of Neural Control, NINCDS). The computations make use of a large electrical network modeling program, "SPICE" which runs on the IBM 370. By adapting our mathematical model to this program, we can specify any combination of electrical membrane properties and any neuron geometry. We have begun to investigate the attenuation and phase shift of sinusoidal current inputs to the soma of a model neuron. The results show the same trends as those observed experimentally in motorneurons of the lobster stomatogastric ganglion. We are currently investigating the effects of including more or less detail in the model to represent the known details of the dendritic branching of these lobster neurons. Consultations have been carried out both with Prof. Gordon Shepherd and with Dr. Robert Burke. The overall objective of these studies is to gain and test specific insights relevant to the integrative properties of dendritic neurons which receive inputs from many afferent sources.

Publications:

Rall, W.: Functional aspects of neuronal geometry, in Neurons without Impulses. Society for Experimental Biology Seminar Series. 6 (Roberts, A. and Bush, B. M. H., eds.), Cambridge Univ. Press, May 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,002-09 MRB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mathematical description of the substrates transport in the capillary-tissue structures.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jose M. Gonzalez-Fernandez	Research Mathematician	MRB, NIADDK
OTHER:	Susie E. Atta	Mathematician	MRB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.0	OTHER: .3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To develop mathematical models of the blood flow and transcapillary exchanges in capillary networks. An effort is being made to incorporate in the models the histological structure of capillary networks as well as different flow patterns from available experimental information. In this model the extraction of substrates with different chemical kinetics at the tissue site will be described. It is expected that this could be used in experimental situations where the extraction of different substrates are measured simultaneously, thus helping to infer the flow pattern features of the microcirculation. In particular a model of the diffusion-consumption of oxygen in striated muscle containing myoglobin (facilitated diffusion) is being developed and pertinent numerical results examined.

The development of a mathematical model to describe the transport of oxygen in solutions of hemoglobin and myoglobin and a numerical method for its solution have been reported previously. The chemical kinetics are described by the single step reaction $P + X \rightleftharpoons PX$. The method is extended to take into account the cooperative properties induced by the four step reaction (Adair) of the hemoglobin-oxygen chemical kinetics. The magnitude of the facilitated transport of oxygen for the model that includes the cooperative properties of hemoglobin is compared with the one for the model associated with the single step reaction. Some features contained in the former that are not available in the single step model are shown to favor the facilitated transport. The results for hemoglobin as it occurs in the red cells make it plausible that facilitation may contribute to the transport of oxygen in the red cell inside the systemic capillaries. A manuscript "Transport of Oxygen in Solutions of Hemoglobin and Myoglobin" is appearing in *Mathematical Biosciences*.

All of the published theoretical studies on facilitated transport have assumed homogeneous spatial properties. In the striated muscle there exist membranes that may impede the diffusion of the carrier myoglobin. A theoretical study is undertaken to analyze the transport in the presence of membranes on the diffusion path. The numerical method above has been extended to include the nonhomogeneous spatial parameters as they occur in the striated muscle. For the numerical computations the values of the parameters were chosen to comprise those of physiological relevance. The numerical results indicate that the presence of membranes tends to decrease the facilitation. For the nonlinear chemical kinetics of oxygen with the carrier, this decrement also depends on the location of the membrane. At the higher oxygen concentration side of the membrane the flow of combined oxygen is transferred to the flow of dissolved oxygen. The reverse process occurs at the lower concentration side. Jump discontinuities on the concentration of the oxygen-carrier compound at the membranes are associated with these transfers. Let Δ^i be absolute value of the jump in the concentration at the i-th membrane. It has shown that the facilitated transport can be represented by

$$F_f = \frac{D_2}{\ell} [x_2^{eq}(x_1^0) - x_2^{eq}(x_1^\ell) - \{\Delta_0^+ + \sum_{i=1}^{n} (\Delta_i^- + \Delta_i^+) + \Delta_{n+1}^-\}] ,$$

where D_2 is the diffusion coefficient of the oxygen-carrier, ℓ is the width of the slab, + represent the higher oxygen concentration side of the membrane, - represent the lower concentration side, n is the number of membranes and $x_2^{eq}(x_1^0)$, $x_2^{eq}(x_1^\ell)$ are the equilibrium concentrations of oxygen-carrier at the faces of the slab. One observes from this expression that the decrement in facilitation depends on the cummulative effect of the jumps. Let σ_i denote the boundary layer width at the i-th membrane. When the intermembrane distance is larger than $2\sigma_i$, it was shown that a relationship exists between Δ^i, the total flow of oxygen and the slope of the oxygen dissociation curve. These relations define an algebraic system whose solution gives the facilitated flow. This algebraic system enables one to find the facilitated flow directly without obtaining the profiles of concentration of the substrates.

Publications:

Gonzalez-Fernandez, J. M. and S. E. Atta: Transport of oxygen in solutions of hemoglobin and myoglobin. Math. Biosci. 54:265-290 (1981).

Gonzalez-Fernandez, J. M. and S. E. Atta: Facilitated transport of oxygen in the presence of membranes on the diffusion path. Biophys. J., (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,004-07 MRB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mathematical description of cellular neuroelectric signal transmission.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Rinzel	Senior Scientist	MRB, NIADDK
OTHER:	William C. Troy	Guest Worker	MRB, NIADDK
	G. Bard Ermentrout	Staff Fellow	MRB, NIADDK

COOPERATING UNITS (if any)

D. Terman, Post-doc Research University of Wisconsin Madison, WI	A. C. Scott, Prof. Elec. Eng. University of Wisconsin Madison, WI

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.7	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This project continues to focus on the formulation, analysis, and biophysical interpretation of mathematical models which describe various aspects of neuroelectric signaling for individual neurons. Among the topics of current interest are:
(i) integration of synaptic input delivered to the soma and dendritic branches of a neuron; (ii) steady propagation of action potentials along axons;
(iii) stimulus-response and threshold properties for repetitive-firing of action potentials.

Mathematical models of these phenomena involve systems of linear and nonlinear ordinary differential equations and parabolic partial differential equations. Solutions and their mathematical stability are determined by analytical and numerical methods. One aspect in the approach of this project is to expose the qualitative mathematical structure for classes of models by exploiting simple, yet reasonable, equations. Because qualititatively related mathematical problems arise in other biological and chemical contexts, e.g. chemical and biochemical oscillations, this project may consider models from such applications.

PHS-6040
(Rev. 2-81)

1. (With W. C. Troy) Various oscillation phenomena of the Belousov-Zhabotinskii chemical reaction are accounted for by Field-Noyes model and its modifications. By considering simplified two or three variable versions of the mathematical model we analyze solutions which represent regular oscillations or excitability in a batch reactor and bursting patterns of oscillations in a flow reactor. To understand bursting we view certain fixed parameters in the batch model as slow dynamic variables in the flow model. In one case, the stoichiometric parameter f slowly sweeps back and forth through values for which the batch model has both a stable steady state and a stable limit cycle. This hysteresis mechanism produces bursts, i.e. alternating periods of oscillations and quiescence. By considering a range of parameter values (e.g. flow rate) we explain other qualitatively different bursting phenomena in terms of the simplified models. These results were presented at the Field Workshop in Mathematical Biology (Utah, June, 1981). A manuscript is now in final preparation. This investigation may provide insight on the mechanism for bursting impulse patterns of certain nerve membrane.

2. (With A. C. Scott) A simple kinematic approximation for the x-t trajectories, and speeds, of propagating nerve impulses has been proposed (R. N. Miller and J. Rinzel, Biophys. J. 34 (1981) 227-259). By using the Hodgkin-Huxley model as a test case for a two-pulse simulation, we are comparing this procedure with other approximate descriptions in the literature. The mechanism for pulse-locking (i.e., the interval between successive pulses asymptotes to a fixed value during propagation) in the latter descriptions appears less applicable. Because the contrast is not overwhelming for this parameter set, we seek other parameters and corresponding experimental conditions to enhance the phenomena of pulse locking, as well as, supernormal condition velocities.

3. Various aspects of mathematical modeling in cellular neurobiology were reviewed and presented at two conferences: 1980 Summer Seminar on Mathematical Aspects of Physiology (Salt Lake City, Utah) and NATO Advanced Study Institute, Nonlinear Phenomena in Physics and Biology (Banff, Canada). A paper, to be published for each conference, includes some recent results on impulse propagation in branching axons, on the onset of repetitive firing as a bifurcation phenomenon, on repetitive bursting neurons as endogenous oscillators, and on bursting patterns induced by network coupling.

4. (With D. Terman) We previously observed, for a bistable FitzHugh-Nagumo reaction-diffusion system, that certain transition-type traveling waves may be reflected by local disturbances and others may not. In an idealized formulation we treat the local disturbance as a specified boundary condition. For some boundary conditions we demonstrate analytically the existence of a stationary solution which is stable. In such a case, we predict and observe numerically that one kind of wave will approach this stationary solution and not be reflected. We conjecture that, for situations which lead to reflection, either there is no stationary solution or the existing stationary solution is unstable. The idealized problem has direct application; e.g. for behavior of a semi-infinite biochemical medium in contact with an infinite bath. A manuscript has been submitted to SIAM Journal of Applied Mathematics.

5. (With G. B. Ermentrout) We have studied dynamic behavior in a simple, one-variable, reaction-diffusion model for the cases of either excitable or oscillatory reaction kinetics (G. B. Ermentrout and J. Rinzel, J. Math. Biology 11 (1981) 269-294). We are now investigating how solutions of the simple phase model are useful for asymptotically solving certain, more complicated, models. For example,

by a singular perturbation procedure, we obtain spatially uniform oscillations and their period for a parameter near its critical value (infinite period case). We also consider temporal phase-locking when such a system near criticality is forced periodically.

Publications:

Guttman, R., S. Lewis, and J. Rinzel: Control of repetitive firing in squid axon membrane as a model for a neuron oscillator. J. Physiol. (Lond.), 305:377-395, 1980.

Ermentrout, G. B. and J. Rinzel: One-dimensional λ-ω target patterns: empirical stability tests. J. Math. Biology 10:97-100, 1980.

Ermentrout, G. B. and J. Rinzel: Waves in a simple, excitable or oscillatory, reaction-diffusion model. J. Math. Biology 11:269-294, 1981.

Miller, R. N. and J. Rinzel: The dependence of impulse propagation speed on firing frequency, dispersion, for the Hodgkin-Huxley model. Biophys. J. 34:227-259, 1981.

Keener, J. P., F. C. Hoppensteadt, and J. Rinzel: Integrate-and-fire models of nerve membrane response to oscillatory input. SIAM J. Appl. Math. (in press).

Rinzel, J.: Models in neurobiology. In Mathematical Aspects of Physiology (ed., F. C. Hoppensteadt), Vol. 19, Lectures in Applied Mathematics, Amer. Math. Soc., Providence (in press).

Rinzel, J.: Models in neurobiology. In Nonlinear Phenomena in Physics and Biology (ed., R. H. Enns), Plenum (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,013-02 MRB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mathematical modeling of biological waves.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Bard Ermentrout	Staff Fellow	MRB, NIADDK
OTHER:	John Rinzel	Senior Scientist	MRB, NIADDK

COOPERATING UNITS (if any)

Dr. Nancy Kopell, Professor Northeastern University Boston, MA	Dr. Barry Bunow DCRT, LAS NIH

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.3	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the formulation, analysis, and interpretation of mathematical models which describe biological waves and oscillations. The main topics of interest are:

(i) neuronal networks and their application to the understanding of various nonlinear neuronal phenomena such as phosphenes, hallucinations, and epilepsy;

(ii) excitable and oscillatory systems and their behavior under periodic forcing;

(iii) general theories of stationary, homogeneous media;

(iv) chains of oscillators useful in modeling intestinal and gastric waves.

Our prime objective is to expose the qualitative behavior of the models as an explanation for observed biological patterns.

(1) We have continued to investigate the properties of stationary, homogeneous media. These systems are representative of a large class of biologically interesting models. The properties of such spatially distributed media are independent of spatial position and time. With the use of group theory and a multiple scale method, we are able to classify the stable small amplitude nonlinear behavior of these systems. In particular, we reduce a possibly infinite dimensional problem to one of finite dimensions which has a simple parabolic form:

$$\frac{\partial z_j}{\partial \tau} = D_j \frac{\partial z_j}{\partial \xi^2} + z_j f(|z_1|,|z_2|, \ldots) .$$

The analysis of this system is straightforward and yields insight into the behavior of the full biological model. In a series of three papers (in final preparation) we have deduced each of several qualitatively distinct behaviors: propagating wavefronts, bulk oscillations, periodic wavetrains, standing waves, and standing spatial patterns. The relevance of these to biological systems is described.

(2) Analysis of neuronal nets in a variety of paradigms has lead to a qualitative explanation of several seemingly diverse phenomena. We have used a simplified cortical network to explain the striking visual patterns obtained when deep pressure is applied to the eyes or when one is subjected to bright flicker. A mathematical analysis of the resultant equations on an infinite planar domain leads to several testable hypotheses. In particular, it is shown that hysteresis between no pattern and hexagonal mosaics exists as well as hysteresis between mosaics and spiral patterns. By varying the frequency or intensity of the flickering light one can jump from one pattern to the next. Our approach utilizes perturbed bifurcation theory and symmetry arguments. Numerical evidence is used to further strengthen our arguments away from the quiet of patterning. This work is presently being written up and was the subject of a talk at the Field Workshop in Mathematical Biology, Salt Lake City, Utah, June, 1981.

A similar neuronal network under different conditions leads to rotating patterns and waves. These waves are qualitatively identical to those observed in multielectrode electrocorticograms. The methods we use enable us to predict qualities such as velocity, wave number, and frequency. This work is in preparation.

(3) (With N. Kopell) A multiscale perturbation method is used to reduce a system of coupled oscillators to N scalar equations on an N-torus. A further reduction allows one to study the global behavior of the oscillators by examining the fixed points of an (N-1)-torus. With techniques developed by Kopell, we are able to demonstrate the existence of an invariant manifold as the fixed points coalesce. The geometry of this system allows one to reinterpret these results in terms of the "average" frequency of the individual oscillators. By applying this technique to models of the large and small intestine, we are able to quantitatively predict the position and depth of the well known but poorly understood frequency plateaus experimentally observed.

(4) A method of multiple scales is applied to weakly two coupled oscillators with widely differing frequencies (but nearly rationally related) we are able to determine conditions for phase locking of various patterns (n:m) as well as the ranges of parameters which allow this. The method is applied to λ-ω systems, forced oscillators, and integrate and fire models. The results will appear in J. Math. Biol., 1981.

(5) A system which has a "degenerate" homoclinic orbit is perturbed. Such systems are interesting since they can be either excitable or oscillatory depending on the

type of perturbation. Given a system:

$$(*)\ u_t = F(u),$$

we say (*) has a degenerate homoclinic orbit if there is a trajectory, $u^0(t) \not\equiv 0$ which satisfies:

$$\lim_{t \to \pm\infty} u^0(t) = 0$$

and $F(0) = 0$, $\det(DF(0)) = 0$. We use matched asymptotic expansions and singular perturbation theory to construct periodic solutions to (S) when it is periodically perturbed. We deduce an equation:

$$v_{tt} = -vQ(t)$$

where v is a scalar and $Q(t)$ is periodic. The behavior of this equation completely describes the behavior of (*) when it is perturbed. This work is in progress.

(6) (With J. Rinzel) We are considering the effect of adding diffusion to (*) with regard to the existence of traveling waves. The work is as yet incomplete, but some promising results have been obtained.

(7) (With B. Bunow) The bifurcation diagram of a certain bound enzyme system has been deduced near a singular point. We find secondary loops and other phenomena which were observed in numerical simulations. Our methods are analytic. The results are being prepared.

Publications:

Ermentrout, G. B. and Rinzel, J.: One dimensional λ-ω target patterns: empirical stability tests. J. Math. Biol. 10:97-100 (1980).

Ermentrout, G. B.: Stable small amplitude solutions in reaction-diffusion systems. Quart. Appl. Math. : 61-86, (1981).

Ermentrout, G. B. and Rinzel, J.: Waves in a simple excitable or oscillatory, reaction-diffusion model. J. Math. Biol. 11:269-294 (1981).

Ermentrout, G. B.: n:m phase-locking in weakly coupled oscillators. J. Math. Biol. (in press).

Annual Report of the Arthritis and Rheumatism Branch

National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases

The Branch program has involved studies ranging from the molecular level through animal model work to clinical studies of systemic lupus erythematosus and rheumatoid arthritis.

The work on IgE receptors of mast cells, considered a model of cell-stimulation upon contact with antigen-antibody complexes, has progressed with the identification of two subunits of the α chain. The α_1 chain is somewhat larger and incorporates carbohydrates; the α_2 chain is implicated as the actual combining site for IgE. The existence of the two subunit tends to confirm the remarkable finding by radiation inactivation target analysis that the binding site is smaller than the mass of the entire α chain. The α chain may be anchored to the membrane via the β chain; hydrophobic domains of the latter are being sought.

Progress has also been made by incorporating the receptors and their complexes in artificial phospholipid liposomes; in studies of receptor phosphorylation upon stimulation of the receptor; and in analyses of changes in the IgE itself upon complexing with the receptor (25).

The internalization of Igs after interacting with cell membrane receptor was found to be driven by the receptor rather than by the nature or degree of polymerization of the Ig. The process occurred equally in cell lines differing in histamine exocytosis upon internalization. Specific antibodies were used to demonstrate that receptors for IgE and for IgG are antigenically distinct molecules. Work in progress with membrane vesicles prepared from rat basophilic leukemia cell suggests unexpectedly that the IgE receptor is not a transmembrane protein (51).

Previous fundamental work with model, covalently linked antigen-antibody complexes permitted the extension of the method to a large, soluble, antigen, the carbohydrate polymer Ficoll. Anti-dinitrophenol antibodies form complexes with dinitrophenol derrivatized Ficoll. The Ficoll backbone was further modified by the addition of particular monosaccharides. It was demonstrated that galactose or mannose derivatized Ficoll in antigen-antibody complex was able to bind to hepatocyte galactose receptors or liver endothelial and Kupffer cell mannose receptors, respectively, in addition to their binding via the antibody immunoglobulin molecule (18). The investigators received the 1981 French Rheumatological Society Prize for this work.

Attention continues to be focussed on the spontaneous diseases developed by various strains of inbred mice, the New Zealand series, MRL/1 and BXSB together with various backcrosses to other strains such as CBA/N and BALB/C. The work with recombinants makes it clear that there is no "autoimmune gene." The full expression of the disease of NZB mice requires at least six separate genes. An X-linked defect of T-independent immune responsiveness of CBA/N has been bred into NZB mice where the defect is full expressed although 90% of the genetic material is NZB; such animals fail to develope anti-DNA antibodies and live a normal life span (20).

Sex hormones, given early in animal life, have major effects on outcome not seen if given later. Their effect may well be in modulating maturational events in the immune system which in turn controls the potential for autoimmunity (21).

Environmental factors also play a role. Sendai virus infection markedly alters NZB disease and sex hormone responsiveness. The injection of polyclonal B cell activators can induce rapidly progressive autoimmune disease and death even in strains not destined to develope autoimmune disease. The same activators, poly rI·rC, for example, induce amyloidosis even in those with the X-linked defect mentioned earlier (20).

Hybridoma technology has been successful in producing anti-DNA and anti-anti-DNA antibodies. The hybridoma cells show remarkably different growth rates with various host strains of mice (21).

Treatment of the mouse diseases has been further examined. MRL/1 mouse disease was greatly alleviated producing increased survival by neonatal thymectomy. With workers in Boston, a diet high in fish oil was found greatly to prolong life in NZB/W mice; the effect is believed to be through the arachidonic acid - prostaglandin pathway (22).

Another animal model, chronic erosive arthritis induced in rats by single intraperitoneal injections of Group A streptoccocal cell walls, has been shown histologically and radiographically closely to mimic rheumatoid arthritis. The differential susceptibility to the arthritis of 16 strains of inbred rats may relate to macrophage function. In resistant strains the macrophages appear to suppress lymphoproliferative responses over prolonged periods whereas in the susceptible, after a lag period, the macrophages, perhaps abetted by T cells, can be shown to enhance lymphoproliferation (48).

Further insight into pathogenetic mechanisms of human SLE has resulted from identifying circulating T-cell subsets with monoclonal antibodies and fluorescence cell sorting. The ratio of suppressor cells to helper cells, varying in large part through changes in numbers of the latter, appears to be related to disease type as well as disease activity. Patients with high suppressor:helper ratios had early onset disease, renal involvement and thrombocytopenia. Those with lower ratios often had myositis, Raynaud's phenomenon and the sicca syndrome (23). Intensive analysis of outcome as related to histopathology of the kidneys in nephritic SLE patients continues (38). Findings relating to the relationship of spontaneously activated B-cells and disease activity in SLE were reported (52).

In the studies of management of SLE, two major milestones were passed. Entry of patients with nephritis into the PACIV study was stopped although followup and reporting of the results both of that study and the even earlier CAP study will continue (00 & 40). Based upon the findings of those studies, the results of others using bolus steroid programs, and some pilot studies of our own, a new program was begun (56). It calls for randomizing patients to monthly steroid bolus or to monthly intravenous cyclophosphamide bolus, the latter representing an accelerated form of a program previously used. The overall approach represents a shift toward intermittent rather than continuous treatment, intensive with very active disease and sharp reductions of medication intake during inactive phases.

The second major accomplishment was the completion of the only fully controlled study of plasmapheresis ever conducted for SLE (55). Patients with relatively mild disease were randomized to and received either bonafide plasmapheresis (12 liters weekly) or a sham procedure for a two week period. The results showed sharp reductions in immune complexes and Ig concentrations in those receiving bonafide treatment. Both groups improved clinically with equal rapidity. The results considerably disturbed the concept that all symptoms and findings in SLE are directly related to the deposition of immune complexes.

The ARB continued to act as one of the cooperating clinics in national studies comparing several modes of therapy for rheumatoid arthritis (49 & 50). Pulse intravenous methylprednisolone therapy has also been assessed and found beneficial; pharmacologic studies of single, large oral doses are now being evaluated with the thought that the same good benefit/risk ratio may be achieved without the technical limitation of parenteral therapy (53).

Lymphapheresis as a treatment mode for RA has been further studied using a two week, six treatment schedule. There are striking divergences in clinical response - a small proportion of active patients achieving early and persistent benefit and a larger group manifesting limited or no improvement. The good responders begin treatment with a greatly elevated and abnormal number of Ia-bearing T-cells, respond poorly to skin test antigens, and have virtually no *in vitro* lymphoproliferative response to soluble antigens; all three of these detected abnormalities return rather dramatically toward normal with the first one or two lymphapheretic treatments. The findings are pushing us toward a more in depth study of lymphocyte and monocyte trafficking in RA (54).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41000-12 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Controlled study of cyclophosphamide and azathioprine in systemic lupus erythematosus with nephritis (CAP)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.L. Decker	Chief, Arthritis and Rheumatism Br.	ARB NIADDK
	A.D. Steinberg	Senior Investigator	ARB NIADDK
OTHER:	P.H. Plotz	Senior Investigator	ARB NIADDK
	J.H. Klippel	Senior Investigator	ARB NIADDK
	J.E. Balow	Senior Investigator	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with diffuse glomerulonephritis and systemic lupus erythematosus satisfying criteria for active renal disease are offered a therapeutic study. The patients are randomized to different treatment programs which include the following drugs: corticosteroids, azathioprine and cyclophosphamide.

Follow-up visits occur every six months and the patients are followed until death or dialysis. Clinical and immunologic assessment are performed. The risks and benefits of each treatment will be evaluated and compared.

Project Description:

Objectives:

Two ten week studies by our Branch suggested that cyclophosphamide was effective in the treatment of lupus nephritis and that azathioprine was less effective. A long-term study of these patients is being carried out to determine whether or not one therapeutic program is superior over the course of several years.

Methods Employed:

Patients meeting predefined criteria for diagnosis of SLE and activity of nephritis have been admitted to the trial. In a baseline control period, the patients were kept on the anti-inflammatory regimen (usually prednisone and aspirin) needed to control extra-renal manifestations. These have been continued with as little change as possible.

A large number of serological, chemical and urine values are serially obtained and the patients will be followed for life. Immunoglobulin levels, anti-DNA antibodies, and serum complement are among those measures. We will determine time to death or renal dialysis in each treatment program and assess the toxicity as well as efficacy of each program.

Major Findings:

The first patient was admitted to this trial in April 1970 and the 38th and last in October 1972.

After courses ranging up to 7 years duration, we were unable to detect an advantage conferred by cyclophosphamide or azathioprine to corticosteroids alone. Furthermore, we were increasingly impressed with the untoward effects of cyclophosphamide, particularly hemorrhagic cystitis and carcinoma of the bladder. In contrast, azathioprine appeared quite safe.

Accordingly, in March 1979 a plan was developed for the discontinuation of these cytotoxic drugs and the intensive observation of study patients during the period following withdrawal. All patients were withdrawn from either cyclophosphamide or azathioprine by December 1979. No obvious exacerbations of SLE were detected as result of drug withdrawal. A final report of the entire study and the withdrawal effects is in preparation.

Significance to Bio-Medical Research and the Program of the Institute:

This work has already had wide medical impact. The project should allow us to draw conclusions in reference to the long-term hazards of drug administration and withdrawal.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41018-07 ARB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Production of covalently cross-linked immune complexes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Plotz	Senior Investigator	ARB NIADDK
OTHER:	D. Finbloom	Clinical Associate	ARB NIADDK
	D. Magilavy	Clinical Associate	ARB NIADDK
	A. Rifai	Staff Fellow	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three major aspects of immune complex biology have been been studied. First, the ability of the mouse liver to handle infused complexes was studied, using site-specifically cross-linked model complexes. The receptors for complexes on Kupffer cells were found to be specific for the immunoglobulin molecule and not other aggregated proteins removed by the same cells. Furthermore, NZB/W mice, with murine lupus, show an impaired ability to inject complexes after they are bound to Kupffer cells. Second, the influence of antigen or the handling of complexes has been studied using pairs of glycoprotein antigens differing only in their carbohydrate side chains but reacting with the same antibodies to form complexes. Desialylated glycoproteins carry complexes to hepatocytes, whereas complexes with the intact protein go to Kupffer cells. Third, the influence of monosaccharides substituted onto the Ficoll backbone of another group site-specifically cross-linking antigens has been studied. Galactose substitution on the antigen carries the stable complexes to hepatocytes, whereas mannose brings them to Kupffer cells.

Project Description:

Objectives:

To study the role of the configuration of immune complexes in determining their pathogenetic properties.

Methods Employed:

Model complexes were made using a family of bivalent or multivalent affinity-labelling antigens. Immune complex handling was studied by in vivo injection or portal vein infusion of complexes. Kupffer and hepatocyte separation were carried out by enzymatic digestion of the liver.

Major Findings:

1. There are immunoglobulin-specific receptors on mouse Kupffer cells.

2. The behavior of these receptors is abnormal in murine lupus.

3. The nature of the antigen in experimental immune complexes has a profound influence on the biological fate of those complexes in the animal.

Significance to Bio-Medical Research and the Program of the Institute:

The results support the possibility that the homing of antigen-antibody complexes to cells or tissues possessing receptors which recognize the antigen may contribute to the abnormal localization of complexes outside the reticuloendothelial system; such abnormal localization is a major feature of many inflammatory illnesses.

Proposed Course:

Alternate substitutions on the antigenic backbone will be assessed in reference to their ability to modify disposition of the antigen-antibody complexes.

Publications:

1. Finbloom,D.S., Magilavy, D.B., Harford, J.B., Rifai, A., Plotz, P.H: The influence of antigen on immune complex behavior in mice. J. Clin. Inv. In press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41020-14 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Pathogenesis of autoimmunity in mice with SLE-like illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB/NIADDK
OTHER:	P. Smathers	Biologist	ARB/NIADDK
	J. Reeves	Chemist	ARB/NIADDK
	E. Raveche	Chemist	LEP/NIADDK
	J. Cowdery	Clinical Associate	ARB/NIADDK
	E. Novotny	Animal Caretaker	ARB/NIADDK
	C. Rubin	Staff Fellow	ARB/NIADDK
	C. Hansen	Geneticist	R/VR
	C. Laskin	Visiting Fellow	ARB/NIADDK
	H. Smith	Clinical Associate	ARB/NIADDK
	J. Roths		The Jackson Laboratory, Bar Harbor, ME

COOPERATING UNITS (if any)
Laboratory of Experimental Pathology, NIADDK
Small Animal Section, VR
The Jackson Laboratory, Bar Harbor, Maine

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.3	4.0	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Autoimmunity in New Zealand mice has a genetic and immune basis; however, expression of severity can be modified by viral and other environmental factors as well as sex hormones. The immunologic factors are complex. These include excessive B-cell activation and abnormal T cell function. Excessive B-cell activation can be modified by introducing the X chromosome of a CBA/N mouse into the NZB autoimmune background. The CBA/N X chromosome, which codes for defects in B-cell maturation (xid), causes a reduction in autoimmunity in F_1 mice, and in congenic mice. The B cell subset absent in CBA/N mice is that which is hyperactive in NZB mice. In contrast, MRL/1 mice produce autoantibodies via a different B cell subset. Administration of polyclonal B-cell activators (poly rI·rC and LPS) bypasses the xid in NZB hybrids and congenic mice in that other subsets of B cells are stimulated to produce autoantibodies. BXSB mice have a Y chromosome-linked gene which accelerates autoimmunity in autoimmune mice, but does not cause it in non-autoimmune mice.

Project Description:

Objective:

New Zealand mice spontaneously develop an autoimmune disease resembling human systemic lupus erythematosus. Early in life they are immunologically hyperactive, become relatively resistant to tolerance induction, and gradually develop antinuclear and other autoantibodies followed by clinical disease. Both humoral and cellular immune defects have been reported and are under active investigation. In addition, the bases for the differences in disease expression in New Zealand mice are being explored. Recently developed MRL/MP-lpr/lpr (MRL/l) and BSXB mice which develop SLE are also being studied.

Methods Employed:

1. Assays for antibodies to RNA and DNA using ^{14}C-labeled or ^{3}H-labeled nucleic acids as ligands and the ammonium sulfate precipitation assay. Assay of antibody forming cells to sheep erythrocytes and pneumococcal polysaccharide by local hemolysis in gel.

2. Immunization of animals with a variety of antigens with and without protein carriers or adjuvants.

3. Assay of antibodies to erythrocytes by passive hemagglutination.

4. Assay of antibodies to DNP-Ficoll by passive hemagglutination with TNP conjugated OX erythrocytes.

5. Assay of antibodies to bovine gamma globulin by solid phase radioimmunoassay.

6. Assay of IgM and total anti-DNA and anti-RNA by solid phase radioimmunoassay.

7. Assay of anti-idiotype antibodies to anti-rI·rC and anti-DNP Ficoll by solid phase radioimmunoassay.

8. Neonatal thymectomy by midline incision and a suction catheter.

9. Transfer of spleen, bone marrow and thymus cells into recipients to evaluate the functional properties of the different cell types.

10. Study of the graft versus host disease inducing properties of NZB lymphoid cells using the Simonsen spleen weight assay and newborn SWISS mice as recipients.

11. Separation of lymphoid cells functionally (corticosteroid, anti-T cell sera, PNA) and physically (sedimentation).

12. Assay of anti-T cell antibodies by release of ^{51}Cr from thymocytes.

13. Evaluation of subpopulations of T-cells using the fluorescence activated cell sorter.

14. Castration of male and female mice at various ages and treatment with sex hormones by either injection of implantation of silastic tubes containing crystaline hormones.

15. Immunization of mice with H-2 incompatible and H-2 compatible cells and studying in vitro regulation of T-cell function: proliferation in response to, and killing of, the H-2 incompatible cells as well as killing of additional target cells. These studies include time course of responsiveness and kinetics of killing during the early phase of the 4-hour cytotoxicity assay (using ^{51}Cr-labeled targets).

Major Findings:

1. NZB mice and their F_1 hybrids produce excessive polyclonal IgM and autoantibodies of both IgM and IgG classes. CBA/N mice and CBA/N mothered F_1 males fail to make antibody to many T-independent antigens and have low levels of serum IgM; further, these mice lack a population of splenic B-cells characterized by a low-to-intermediate density of surface IgM. The CBA/N X-linked defect of T-independent immune response is completely expressed in NZB.CBA/N congenic mice. These are >90% NZB but all X-chromosomes are from CBA/N mice. Such CBA/N X-chromosome bearing NZB mice fail to make anti-DNA, anti T cell antibodies, and anti-RBC antibodies. They live a normal life span. In contrast, MRL/1 mice bearing the CBA/N X-chromosome make anti-DNA.

2. NZB.CBA/N congenic mice, which do not have autoantibodies and which do not get sick, manifest the T cell defects seen in NZB mice. This result suggests that the T cell defects do not result from the B cell hyperactivity.

3. Androgens promote tolerance in reconstituted (NZB x DBA/2) F_1 mice.

4. Thymocyte subpopulations are modified by sex hormones. Neonatal androgens lead to a resistance to the effects of estrogens administered after two weeks of age.

5. NZO mice develop features of both systemic lupus and diabetes mellitus.

6. Sendai virus markedly alters both the natural history of NZB mice and their responses to sex hormones. A variety of environmental factors are capable of altering the natural history of autoimmune mice.

7. Mice not destined to develop overt clinical autoimmune disease develop rapidly progressive autoimmune disease and premature death when administered polyclonal B cell activators.

8. Polyclonal B-cell activators induce amyloidosis. This occurs in mice bearing CBA/N X-chromosomes indicating that the B-cell subset controlled by xid is not necessary for amyloid production.

9. Analysis of recombinant inbred mice produced by mating NZB with non-autoimmune mice indicates that in NZB mice there is no "autoimmunity gene", rather, the full expression of illness requires at least six separate genes. However, the presence of fewer genes allows for the expression of milder degrees of autoimmunity.

10. Anti-antibodies are produced during a normal primary immune response. Such antibodies play a role in immune regulation; however, antibody is quantitatively more important than anti-antibody in such regulation.

Proposed Course:

The role of genetic factors will be further investigated. This will include further study of congenic and recombinant inbred lines. The availability of athymic nude mice will allow us to study lack of thymic function in different strains with and without superinfection with different viruses or immunization with nucleic acid antigens. We hope thereby to define the relative contributions of genetic and different immunologic factors in the development of autoimmunity. The role of anti-idiotypic antibodies and antibody itself in control of antibody and autoantibody production will be investigated further in autoimmune mice.

Significance to Bio-medical Research and the Program of the Institute:

1. The genetic basis for autoimmunity is becoming much better established. The studies of B-cell defective mice point to potential genetic manipulations.

2. Study of autoimmune mice indicates that there are different cellular bases for SLE. This may lead to different treatment for different patients in the future.

3. Environmental agents can trigger SLE in susceptible individuals that would otherwise not get it.

Publications:

1. Taurog, J.D., Smathers, P.A., Steinberg, A.D.: Evidence for abnormalities in separate lymphocyte populations in NZB mice. J. Immunol. 125:485-490, 1980.

2. Steinberg, A.D., Roths, J.B., Murphy, E.D., Steinberg, R.T., Raveche, E.S.: Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. J. Immunol. 125:871-873, 1980.

3. Glimcher, L.H., Steinberg, A.D., House, S.B., Green, I.: The autologous mixed lymphocyte reaction in strains of mice with autoimmune disease. J. Immunol. 125:1832-1838, 1980.

4. Huston, D.P., Steinberg, A.D.: Kinetic analysis of NZB cytotoxic lymphocyte responses. J. Exp. Med. 152:748-753, 1980.

5. Steinberg, A.D.: "The balance between helper and suppressor factors." In, Strategies of Immune Regulation, Sercarz, E.E., and Cunningham, A.J. (Eds.). Academic Press, New York, 1980, p. 377.

6. Steinberg, A.D.: "Autoimmunity." In, Strategies of Immune Regulation, Sercarz, E.E., and Cunningham, A.J. (Eds.). Academic Press, New York, 1980, p.503.

7. Steinberg, A.D., Smathers, P.A., Boegel, W.A.: Effects of sex hormones on autoantibody production by NZB mice, and modification by environmental factors. Clin. Immunol. Immunopathol. 17:562-572, 1980.

8. Melez, K.A., Harrison, L.C., Gilliam, J.N., Steinberg, A.D.: Autoimmunity in the NZO (New Zealand Obese) mouse. Diabetes 29:835-840, 1980.

9. Steinberg, A.D., Taurog, J.E., Cowdery, J.S., Jr., Huston, D.P., Smathers, P.A., Reeves, J.P., Hansen, C.T., Raveche, E.S.: "Cellular basis of SLE." In, Immunoregulation and Autoimmunity. International Symposium on Autoimmunity. Edited by R. Krakauer and M. Cathcart. Elsevier-North Holland Publishers, New York, 1980, pp. 193-207.

10. Cowdery, J.S., Jr., Taurog, J.D., Steinberg, A.D.: Effect of CBA/N xid on spontaneous production of antibodies to DNA in MRL/1 and NZB backcross mice. Scandinavian J. Immunol. 12:499-502, 1980.

11. Steinberg, A.D., Huston, D.P., Taurog, J.D. Cowdery, J.S., Raveche, E.S.: The cellular and genetic basis of murine lupus. Immunological Reviews 55:121-154, 1981.

12. Taurog, J.D., Raveche, E.S., Smathers, P.A., Glimcher, L.H., Huston, D.P., Hansen, C.T., Steinberg, A.D.: Studies of the pathogenesis of autoimmunity in NZB mice. Demonstration of independent B and T cell abnormalities and prevention of autoimmunity by deletion of a B cell subset. J. Exp. Med. 153:221-234, 1981.

13. Steinberg, A.D., Taurog, J.D., Raveche, E.S., Cowdery, J.S., Smathers, P.A., Reeves, J.P.: The pathogenesis of autoimmunity in New Zealand mice. VI. Balance between genetic and environmental factors with special emphasis upon B-cells. In, B Lymphocytes in the Immune Responses, Functional, Developmental, and Interactive Properties. Proc. Second International Conference on B Lymphocytes in the Immune Response. ed. N. Klinman, D.E. Mosier, I. Scher, E.S. Vitetta. Elsevier-North Holland, New York, 1981, pp. 345-354.

14. Cowdery, J.S., Steinberg, A.D.: Serum antibody binding antibodies produced during a primary immune response. J. Immunol. 126:2136-2138, 1981.

15. Raveche, E.S., Novotny, E.A., Hansen, C.T., Tjio, J.H., Steinberg, A.D.: Genetic studies in NZB mice. V. Recombinant inbred lines demonstrate that separate genes control autoimmune phenotype. J. Exp. Med. 153:1187-1197, 1981.

16. Reeves, J.P., Taurog, J.D., Steinberg, A.D.: Polyclonal B-cell activation of autoantibodies in (CBA/N x NZB) F_1 mice by polyinosinic·polycytidylic acid. Clin. Immunol. Immunopathol. 19:170-180, 1981.

17. Attallah, A.M., Urrita-Shaw, A., Khalil, R.Y., Steinberg, A.D., Ahmed, A.: Interferon immunoregulation of antibody response in normal and autoimmune mice is mediated by suppressor T cell subsets. Int. Archs. Appl. Immunol. 64:353-358, 1981.

18. Cowdery, J.S., and Steinberg, A.D.: Murine models of systemic lupus erythematosus. Adv. Inflam. Res. 2:73-93, 1981.

19. Taurog, J.D., Steinberg, A.D.: Genetic and Immune Aspects of SLE: Recent Advances. Dermatology 20:149-158, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41021-13 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Antinuclear antibodies in spontaneous and drug-induced SLE and other diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
OTHER:	J.S. Cowdery	Clinical Associate	ARB NIADDK
	E.S. Raveche	Chemist	LEP NIADDK
	J.P. Reeves	Chemist	ARB NIADDK
	P.A. Smathers	Biologist	ARB NIADDK
	H.R. Smith	Clinical Associate	ARB NIADDK

COOPERATING UNITS (if any)

Laboratory of Experimental Pathology, NIADDK

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.4	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Antibodies to nucleic acids are important diagnostically and pathogenetically in systemic lupus. The production of these antibodies is in part controlled by the X-chromosome through regulation of maturation of B cells destined to make IgM antibodies to nucleic acids. Sex chromosomes indirectly influence the magnitude to auto-antibody production through sex hormones: male sex hormones suppress the production of anti-nucleic acid antibodies.

Hybridoma cells producing anti-nucleic antibodies (derived originally by fusing BALB/c and NZB x NZW F_1 cells) preferentially grow and preferentially produce different autoantibodies in different recipient strains. These studies suggest that control mechanisms exist for the proliferation of, and perhaps synthesis by, B cells genetically destined to produce antibodies to nucleic acids. Antibodies specific for anti-RNA or anti-DNA antibodies can be produced and used to study the regulation of autoantibody production.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

Study the characteristics of a variety of antinuclear antibodies in human and murine "autoimmune diseases." Determine genetic contributions to the immune response to nucleic acid and related antigens.

Methods Employed:

Farr ammonium sulfate precipitation assay using radiolabeled ligands. Standard precipitin reaction using labeled and unlabeled nucleic acids or nuclear extracts. Hemagglutination, complement fixation and immunodiffusion assays. Standard immunization procedures. Solid phase radioimmunoassay using labelled antibodies to mouse immunoglobulin or classes of immunoglobulins. Standard hybridoma technology.

Major Findings:

1. Genetic studies of NZB x DBA F_1 and backcross mice indicate that a single gene locus (expressing itself in a gene dosage fashion) plays the major role in controlling production of anti-DNA.

2. Analysis of 27 recombinant inbred lines points to a single NZB gene, independent of the gene for anti-T cell antibodies, which is responsible for anti-DNA. This trait was found in about half of the lines.

3. Castration of male NZB x DBA or DBA x NZB mice led to accelerated anti-DNA production, both following immunization and spontaneously. This was suppressed by testosterone and further accelerated by estradiol. Castration of 3 week old NZB/NZW males accelerates anti-DNA production; castration at 3 months does not. Testosterone treatment of 3 week NZB/NZW females retards anti-DNA production; treatment at 3 months suppresses antibodies to ssDNA, but not antibodies to native DNA.

4. NZO mice develop both anti-DNA antibodies and diabetes.

5. Hybridomas produced by fusion of BALB/c-derived myeloma cells (non-producers) with NZB x NZW F_1 spleen cells produce antibodies to nucleic acids _in vitro_ and when transferred to NZB x NZW F_1 recipients. Such hybridomas show preferential growth in different strains and preferential production of anti-nucleic acid antibodies with certain specificities.

6. Antibodies have been produced to the anti-DNA of (NZB x NZW) F_1 and MRL/Mp-_lpr_/_lpr_ mice.

7. The growth of hybridomas producing anti-DNA has unusual strain specificity.

Significance to Bio-Medical Research and the Program of the Institute:

Male sex hormones appear to suppress auto-antibody production explaining the more severe disease and greater incidence in females. This effect occurs primarily very early in life; it suggests that hormones modulate a maturational event in the immune system which in turn regulates autoimmune potential. Genes for high and low responsiveness to DNA may be one of the genetic contributions of autoimmunity in SLE.

Proposed Course:

Further define the genetics of the antibody response to DNA and try to correlate with other immune abnormalities. Further study the characteristics of antinuclear antibodies. Continue to evaluate the role of sex hormones in autoimmunity. Compare antinuclear antibodies of patients with different autoimmune diseases. Further study subpopulations of patients with a high incidence of autoimmunity. Define the role of anti-idiotype antibodies in the regulation of anti-DNA production.

Publications:

1. Taurog, J.D., Smathers, P.A., Steinberg, A.D.: Evidence for abnormalities in separate lymphocyte populations in NZB mice. J. Immunol. 125:485-490, 1980.

2. Steinberg, A.D., Roths, J.B., Murphy, E.D., Steinberg, R.T., Raveche, E.S.: Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. J. Immunol. 125:871-873, 1980.

3. Blaese, R.M., Grayson, J., Steinberg, A.D.: Elevated immunoglobulin secreting cells in the blood of patients with active systemic lupus erythematosus: correlation of laboratory and clinical assessment of disease activity. Am. J. Med. 69:345-350, 1980.

4. Steinberg, A.D., Smathers, P.A., Boegel, W.A.: Effects of sex hormones on autoantibody production by NZB mice, and modification by environmental factors. Clin. Immunol. Immunspathol. 17:562-572, 1980.

5. Melez, K.A., Harrison, L.C., Gilliam, J.N., Steinberg, A.D.: Autoimmunity in the NZO (New Zealand Obese) mouse. Diabetes 29:835-840, 1980.

6. Steinberg, A.D., Taurog, J.D., Cowdery, J.S., Jr., Huston, D.P., Smathers, P.A., Reeves, J.P., Hansen, C.T., Raveche, E.S.: "Cellular basis of SLE." In, Immunoregulation and Autoimmunity. International Symposium on Autoimmunity. Krakauer, R., and Cathcart, M. (Eds.). Elsevier-North Holland Publishers, New York, 1980, pp. 193-207.

7. Cowdery, J.S., Jr., Taurog, J.D., Steinberg, A.D.: Effects of CBA/N xid on spontaneous production of antibodies to DNA in MRL/1 and NZB backcross mice. Scandinavian J. Immunol. 12:499-502, 1980.

8. Steinberg, A.D., Huston, D.P., Taurog, J.D., Cowdery, J.S., Raveche, E.S.: The cellular and genetic basis of murine lupus. Immunological Reviews 55:121-154, 1981.

9. Reeves, J.P., Taurog, J.D., Steinberg, A.D.: Polyclonal B-cell activation of autoantibodies in (CBA/N x NZB) F_1 mice by polyinosinic·polycyticylic acid. Clin. Immunol. Immunopathol. 19:170-180, 1981.

10. Taurog J.D., Raveche E.S., Smathers, P.A., Glimcher L.H., Huston, D.P., Hansen, C.T., Steinberg, A.D.: Studies of the pathogenesis of autoimmunity in NZB mice. Demonstration of independent B and T cell abnormalities and prevention of autoimmunity by deletion of a B cell subset. J. Exp. Med. 153:221-234, 1981.

11. Raveche, E.S., Novotny, E.A., Hansen, C.T., Tjio, J.H., Steinberg, A.D.: Genetic studies in NZB mice. V. "ecombinant inbred lines demonstrate that separate genes control autoimmune phenotype. J. Exp. Med. 153:1187-1197, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41022-10 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Therapeutic Studies in New Zealand Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
	J.P. Reeves	Chemist	ARB NIADDK
	W.A. Boegel	Lab Technician	ARB NIADDK
	P.A. Smathers	Biologist	ARB NIADDK
	D. Robinson		Harvard Medical School
	J. Prickett		Harvard Medical School

COOPERATING UNITS (if any)

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Treatment of NZB/NZW mice with Ribavirin, a broad spectrum antiviral drug, has been very effective. Renal deposits of immune complexes can be prevented. Such treatment is potentially applicable to humans with SLE.

Male sex hormones, testosterone and 5 alpha-dihydrotestosterone (but not Danazol) prolonged life in female NZB/NZW mice. Such treatment was effective even when started after clinical illness and did not require castration for its full effect. However, NZB mice are much less sensitive to androgen administration.

A diet enriched in a polyunsaturated fatty acid markedly retards the development of autoimmunity in (NZB x NZW) F_1 females. Thymectomy markedly retards autoimmunity in MRL/lpr/lpr mice.

Project Description:

Objectives:

NZB/NZW F_1 mice spontaneously develop antibodies to nucleic acids and die of immune complex glomerulonephritis. NZB mice develop Coomb's positive hemolytic anemia, membranous nephrosis and excessive lymphoreticular proliferation. MRL/Mp-lpr/lpr mice develop marked lymphadenopathy antibodies to DNA and glomerulonephritis. BXSB mice, especially males, develop vasculitis, antinuclear antibodies, and glomerulonephritis. These mice are excellent models for comparing different immunosuppressive and anti-viral drug programs. In addition, as the immunologic basis for the disease becomes better understood, they offer a model for studying more specific immunotherapy.

Methods Employed:

1. Comparison of different immunosuppressive and anti-viral drug regimens in natural history of NZB/NZW mice by study of antibodies to DNA, proteinuria and survival.

2. Treatment with suppressor cells and/or suppressor substances derived from such cells in an attempt at restoring normal regulatory functions and improve the natural history of New Zealand mice.

3. Treatment of NZB/NZW, NZB and MRL/Mp-lpr/lpr mice with androgens.

4. Thymectomy.

Major Findings:

1. Androgens prolong life in MRL/Mp-lpr/lpr and female NZB/NZW mice. Both testosterone and dihydrotestosterone were therapeutically effective in a dose-dependent manner. Danazol was of no benefit.

2. NZB mice had a transient reduction in anti-RBC and anti-DNA antibodies with androgen treatment, but no increase in survival.

3. MRL/Mp-lpr/lpr mice had amelioration of disease by neonatal thymectomy. They had a decrease in anti-DNA, proteinuria and a marked increase in survival. Their lymphadenopathy was almost completely prevented.

4. A diet enriched in fish oil containing a polyunsaturated fatty acid markedly prolonged life in (NZB x NZW) F_1 mice.

Significance to Bio-Medical Research and the Program of the Institute:

The mice continue to provide a testing-ground for promising new treatments prior to assessing their effects in patients.

Proposed Course:

Further study of sex hormones in other SLE strains of mice, especially MRL and F_1 crosses of BXSB mice with other strains to determine whether or not such treatment is uniformly effective.

Publications:

1. Steinberg AD: "Immunoregulatory drugs." Chapter 55. In Kelly, W.N., Harris, E.D., Ruddy, S., and Sledge, C.B. (Eds.): Textbook of Rheumatology. Philadelphia, W.B. Saunders Co., 1980, pp. 841-861.

2. Steinberg AD: "Management of systemic lupus erythematosus." Chapter 70. In Kelley, W.N., Harris, E.D., Ruddy, S., and Sledge, C.B. (Eds.): Textbook of Rheumatology. Philadelphia, W.B. Saunders Co., 1980, pp. 1133-1150.

3. Steinberg, A.D., Melez, K.A., Klassen, L.W., Williams, G.W., Reinertsen, J.L., Reeves, J.P., Boegel, W., Smathers, P.A., Gerber, N.L., Duvic, M., Raveche, E.S.: "Newer approaches to the treatment of systemic lupus through study of New Zealand mice." Japan Medical Research Foundation, Editors. Proc. International Symposium on SLE, Kyoto, Japan, Nov. 1978. University of Tokyo Press, 1980, pp. 389-397.

4. Steinberg, A.D., Roths, J.B., Murphy, E.D., Steinberg, R.T., Raveche, E.S.: Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. J. Immunol. 125:871-873, 1980.

5. Steinberg, A.D., Klassen, L.W., Williams, G.W., Reinertsen, J.L., Gerber, N.L.: "Ribavirin treatment of murine systemic lupus erythematosus." In, Ribavirin: A Braod Spectrum Antiviral Agent. Smith, R.A., and Kirkpatrick, W. (Eds.). Academic Press, New York, 1980. pp. 201-211.

6. Steinberg, A.D., Smathers, P.A., Boegel, W.A.: Effects of sex hormones on autoantibody production by NZB mice, and modification by environmental factors. Clin. Immunol. Immunopathol. 17:562-572, 1980.

7. Taurog, J.D., Raveche, E.S., Smathers, P.A., Glimcher, L.H., Huston, D.P., Hansen, C.T., Steinberg, A.D.: Studies of the pathogenesis of autoimmunity in NZB mice. Demonstration of independent B and T cell abnormalities and prevention of autoimmunity by deletion of a B cell subset. J. Exp. Med. 153:221-234, 1981.

8. Cowdery, J.S., Steinberg, A.D.: Serum antibody binding antibodies produced during a primary immune response. J. Immunol. 126:2136-2138, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41023-07 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Immune Abnormalities in Patients with Systemic Lupus Erythematosus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
OTHER:	J.P. Reeves	Chemist	ARB NIADDK
	I. Green	Senior Investigator	LCI NIAID
	R.M. Blaese	Senior Investigator	IB NCI
	J. Grayson	Clinical Associate	IB NCI
	S. Kumagai	Visiting Fellow	LCI NIAID
	J. Smolen	Visiting Fellow	ARB NIADDK
	C. Morimoto	Harvard Medical School, Boston	
	T. Chused	Senior Investigator	LMI NIAID

COOPERATING UNITS (if any)

Immunology Branch, NCI
Laboratory of Clinical Investigations, NIAID

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Excessive B cell activity and loss of regulatory cells occurs in patients with active SLE; these abnormalities are less marked when the disease is quiescent. Nevertheless, excessive B cell proliferation occurs even in inactive patients.

Loss of a subpopulation of regulatory T cells occurs in patients with active SLE. Antibodies to the precursors of suppressor T cells are spontaneously produced by the patients. Such antibodies prevent normal T cell control of abnormal B cell activity allowing perpetuation of autoantibody production. Studies with monoclonal anti-T cell antibodies indicate that many patients have abnormal presence of activated T cells and increased numbers of B cells. Study of a large number of patients with SLE indicates that SLE is not a disease, but a syndrome with different cellular bases.

Project Description:

Objectives:

To apply the newer understanding of basic immunologic principles, and observations in mice who develop lupus-like diseases, to the study of patients with SLE.

Methods Employed:

1. Study of families of patients with SLE or larger groups of patients with a high incidence of SLE. A battery of immunologic measurements would be made in such patients.

2. Study of lymphocytes and antibodies to lymphocytes from patients with SLE.

   a. Number and distribution of T, B and null cells and their responses to mitogens or allogeneic cells.

   b. Study of antibody producing plaque forming cells (PFC) in the peripheral blood of patients.

   c. Study of generation of suppressor cells by activating purified T cells with concanavalin A and adding them to a second culture, or by adding cells directly to cultures.

   d. Study of other T cell-B cell interactions with the autologous MLR.

   e. Study of anti-lymphocyte antibodies by use of the fluorescence activated cell sorter (FACS). Additional analyses use monoclonal anti-T cell antibodies.

   f. Study of anti-lymphocyte antibodies by complement-mediated lysis, dye exclusion, and antibody dependent direct cellular cytotoxicity (ADCC).

   g. Use of the technique of flow microfluorometry to analyze lymphocyte subpopulations reactive with a variety of specific monoclonal antibodies.

Major Findings:

1. Anti-lymphocyte antibodies occur in association with increased activity of patients with SLE. These antibodies can reduce the number of peripheral T cells and interfere with their function.

2. Antibodies to T cells present in the plasma of patients with active systemic lupus erythematosus (SLE) plus complement are able to eliminate a T cell necessary for the generation of suppressor cells, rather than mature, activated suppressor cells. Such cells are also eliminated by antibody dependent direct cellular cytotoxicity (ADCC). Cells which form rosettes with human erythrocytes are preferentially eliminated. Although the rosetting is not specific for autologous erythrocytes, those cells are

enriched in cells necessary for suppressor function. Such observations suggest that some T cell defects associated with active SLE may not be intrinsic T cell abnormalities, but, rather, secondary effects of anti-T cell antibodies.

3. Patients with active SLE have increased quantities of antibody producing PFC to defined chemical haptens.

4. Using the reverse plaque assay, active SLE was found to correlate with IgG antibody producing cells better than with anti-DNA, C3, and other typical serologic measures. Of interest, serum IgG concentrations correlated very well with active SLE.

5. Studies with monoclonal antibodies, which identify T cell subsets, indicate that some patients with active SLE have a marked decrease in suppressor cells and suppressor cell precursors. Other patients have an increase in suppressor cells relative to helper cells. In most cases of abnormal T suppressor/T helper ratios, the abnormal ratio stems from a quantitative defect in helper cells rather than suppressor cells. Most importantly, the ratio correlated with clinical features of SLE. High suppressor : helper ratios are associated with renal disease, thrombocytopenia, and SLE onset early in life ($\leq$ 20 years). In contrast, patients with relatively fewer suppressors than normal tend to have a multisystem disease with muscle disease, Raynaud's phemenon, and the sicca syndrome.

Significance to Bio-medical Research and the Program of the Institute:

The studies to date suggest that patients with SLE have generalized B cell hyperactivity rather than selective B cell hyperactivity towards self-antigens. However, both genetic factors and environmental exposure determine which B cell clones will be expanded. Loss of suppressor and helper cells in human SLE is associated with (a) disease activity, and (b) anti-T-cell antibodies. These defects preferentially occur during disease activity, indicating that the abnormalities are not permanent, and that appropriate specific prevention may be possible.

The association of clinical features with the ratio of suppressor to helper cells suggests that SLE may have a different cellular basis in different patients. Thus, SLE may not be a disease entity, but a clinical syndrome with different causes. This would direct further study of pathogenesis and therapy at patient subgroups.

Proposed Course:

Continue the studies initiated.

Publications:

1. Blaese, R.M., Grayson, J., Steinberg, A.D.: Elevated immunoglobulin secreting cells in the blood of patients with active systemic lupus erythematosus: correlation of laboratory and clinical assessment of disease activity. Am. J. Med. 69:345-350, 1980.

2. Steinberg, A.D.: "The balance between helper and suppressor factors." In, Strategies of Immune Regulation, Sercarz, E.E., and Cunningham, A.J. (Eds.). Academic Press, New York, 1980, p. 377.

3. Steinberg, A.D.: "Autoimmunity." In, Strageties of Immune Regulation, Sercarz, E.E., and Cunningham, A.J. (Eds.). Academic Press, New York, 1980, p. 503.

4. Lewis, V.M., Twomey, J.J., Steinberg, A.D., Goldstein, G.: Serum thymic hormone activity with systemic lupus erythematosus. Clin. Immunol. Immunopathol. 18:61-67, 1981.

5. Steinberg, A.D., Taurog, J.D., Cowdery, J.S., Jr., Huston, D.P., Smathers, P.A., Reeves, J.P., Hansen, C.T., Raveche, E.S.: "Cellular basis of SLE." In, Immunoregulation and Autoimmunity. International Symposium on Autoimmunity. Krakauer, R., and Cathcart, M. (Eds.) Elsevier-North Holland Publishers, New York, 1980, pp. 193-207.

6. Morimoto, C., Reinherz, E.L., Schlossman, S.F., Schur, P.H., Mills, J.A., Steinberg, A.D.: Alterations of immunoregulatory T cell subsets in active systemic lupus erythematosus. J. Clin. Invest. 66:1171-1174, 1980.

7. Williams, G.W., Bluestein, H.G., Steinberg, A.D.: Brain reactive lymphocytotoxic antibody in the cerebrospinal fluid of patients with systemic lupus erythematosus: Correlation with central nervous system involvement. Clin. Immunol. Immunopathol. 18:126-132, 1981.

8. Morimoto, C., Reinherz, E.L., Borel, Y., Mantzouranis, E., Steinberg, A.E., Schlossman, S.F.: An autoantibody to an immunoregulatory inducer population in patients with juvenile rheumatoid arthritis. J. Clin. Invest. 67:753-761, 1981.

9. Kumagai, S., Steinberg, A.D., Green, I: Antibodies to T cells in patients with SLE mediated ADCC against human T cell. J. Clin. Invest. 67:605-614, 1981.

10. Steinberg, A.D.: Modulation of a complex immune system. Ann. Neurology 9 suppl:117-124, 1981.

11. Hirata, F., del Carmine, R., Nelson, C.A., Axelrod, J., Schiffman, E., Warabi, A., De Blas, A.L., Nirenberg, M., Manganiello, V., Vaughan, M., Kumagai, S., Green, I., Decker, J.L., Steinberg, A.D.: Presence of autoantibody for phospholipase inhibitory protein, lipomodulin, in patients with rheumatic diseases. Proc. Nat. Acad. Sci. (in press).

12. Taurog, J.D. Steinberg, A.D.: Genetic and immune aspects of SLE: Recent advances. Dermatology 20:149-158, 1981.

13. Bluestein, H.G., Williams, G.W., Steinberg, A.D.: Cerebrospinal fluid antibodies to neuronal cells: Association with neuropsychiatric manifestations of systemic lupus erythematosus. Amer. J. Med. 70:240-246, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-41025-10 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on the cell surface receptor for IgE

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Metzger	Chief, Sec. Chem. Immunol.	ARB/NIADDK
OTHER:	C. Fewtrell	Visiting Scientist	ARB/NIADDK
	A. Goetze	Guest Worker	ARB/NIADDK
	B. Rivnay	Visiting Fellow	ARB/NIADDK
	R. Perez	Guest Worker	ARB/NIADDK
	S. Wank	Research Associate	ARB/NIADDK
	D. Finbloom	Clinical Associate	ARB/NIADDK
	E. Kempner	Senior Investigator	LPB/NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 7	PROFESSIONAL: 6	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are pursuing an in-depth investigation on the structure and function of the membrane receptor for IgE. For these studies intact cells from a rat basophilic leukemia as well as normal rat mast cells are being used. In addition receptor-bearing membrane vesicles and solubilized receptors are being studied. The aim is to understand the structure of the receptor as well as its integration in the plasma membrane. Results of such studies as well as direct functional studies are aimed at understanding the early stimulation of cells by antigen-induced, antibody-mediated reactions.

Project Description:

Objectives:

IgE is a unique class of immunoglobulin which plays a predominant role in the physiological events associated with a variety of allergic phenomena. The immunoglobulin becomes attached to the surface of basophils and mast cells and, upon exposure to the allergenic antigen, mediates the release of histamine and other vasoactive amines from the cells to which it is attached. Although there is an increasing amount of information concerning the biochemistry of this reaction almost nothing is known about how the IgE acts as the cell surface transducer. The present project is directed towards increasing our understanding of how the IgE interacts with mast cells. In addition we wish to understand the mechanism by which the ternary complex of antigen-IgE antibody-cell surface receptor triggers the degranulation of basophilic cells.

Methods Employed:

No unusual methods not previously described were employed during the past year.

Major Findings:

Since our previous report the following has been found:

A. Functional Studies:

1. Structural Studies on Receptor

   1. The principal new observations relate to the intra-subunit structure of the α-chain of the receptor. We previously reported that the 50K "α" subunit yielded a 30K fragment exclusively during the early stages of proteolytic digestion in ionic detergent. This proved to be true regardless of whether the α chains were labeled extrinsically (e.g. iodination by Bolton-Hunter reagent) or intrinsically (by isolation from cells grown in the presence of ^{3}H-amino acids). The latter new findings indicated that the 30K digestion product must represent two relatively stable domains roughly equivalent in size. Differential labeling studies substantiated this proposal. We were able to discriminate between the two domains: α 1 behaves as a somewhat larger fragment and incorporates more carbohydrate than α 2. The latter appears to be the principal region of the α chain which becomes modified by surface labeling techniques and is therefore implicated as containing the combining site for IgE.

   2. Since previous studies had indicated that the α chain might be anchored in the membrane via the β-chain we have initiated studies to determine to what extent the chain has a hydrophobic

domain. We have found that the isolated IgE-α chain complex is completely soluble even in the absence of detergent, and are presently studying whether it binds detergent. 'Charge shift' electrophoresis studies have so far given ambiguous results as have studies using the hydrophobic probe iodonaphthylazide. Finally whereas the intact IgE-receptor complex (IgE-$\alpha\beta$) appears to incorporate into liposomes, the IgE-α chain apparently does not (below).

3. We previously reported that target analysis by radiation inactivation suggested that the functional target of α chains (for binding IgE) was smaller than the mass of the α chain as determined by structural analysis. The clarification of the sub-structure of the α-chain (above) tended to confirm this unprecedented observation. Further evidence in favor of the proposal that radiation inactivation might reveal intra-polypeptide functional units was obtained by examining IgE. Again we found that the functional target (for binding to the receptor for IgE) was substantially smaller than the smallest covalently bonded unit (117,000 vs 200,000).

4. During previous studies on the β subunit of the receptor we found that the latter chain gradually dissociates from α even in the absence of overt denaturants. We are intensively engaged in working out procedures by which the two chains may be kept associated during purification. The ability to cross-link the chains is being used as an assay.

B. Reincorporation Studies

A variety of structural and functional questions can be best answered by studying purified receptors which have been reincorporated into phospholipid liposomes. Over the past year we have made considerable progress in obtaining such preparations. We have found that purified IgE-α chain complexes incorporate only poorly but that partially purified intact receptors (IgE-$\alpha\beta$) incorporate rather well. Incorporation is highly sensitive to detergent:lipid ratios. Other parameters (lipid:protein ratio, temperature, type of detergent, rate of detergent removal, etc.) are being explored to determine the optimal conditions.

C. Phosphorylation Studies

A project which we have continuously pursued is to look for changes in the receptor for IgE which might occur as a consequence of triggering. The latest endeavor has been to see if the receptor incorporates radioactive phosphorus and whether there are changes when the receptors are stimulated. We have found that the β chain (but not the α chain) is phosphorylated when cells are exposed to ^{32}P orthophosphate. Incorporation does not appear to be affected by the presence or absence of IgE, and so far no changes resulting from receptor aggregation (which causes stimulation of the cells) have been observed.

D. Studies on IgE.

We have repeatedly attempted to generate an Fc fragment from rat IgE which would continue to show binding activity for the receptor. In our hands (and in other laboratories) this has not been possible. The availability of a mouse IgE hybridoma and purified IgE-α chain complexes has encouraged us to try again. We have found that the mouse IgE unfortunately presents the same difficulties as does the rat IgE. However, during the course of this work we found that during the early stages of digestion by IgE-α chain complexes, the IgE becomes 'nicked' at 2 sites in the epsilon (ε) chains. The pieces appear to remain attached to each other by disulfide bounds since reduction of the "nicked" molecules leads to fragmentation of the ε chains. The nicked molecules appear to retain binding activity for the receptor and under these conditions the α chains themselves appear not to be digested. These preparations of nicked IgE-α chain complexes may be useful in obtaining receptors which can be purified and free of IgE without the use of strong denaturants.

Significance to Bio-Medical Research and the Program of the Institute:

The IgE Mast cell system is of significance in the pathophysiology of allergy, parasitic infections, and inflammation. It is also an experimentally convenient system to study mechanisms of antigen-induced antibody-mediated cellular reactions in general. Finally it is an excellent model for the study of exocytosis. So far we are continuing to make good progress in the elucidation of the molecular mechanisms of this system. Knowledge of these may eventually permit rational intervention in situations where the system contributes to pathology.

Proposed course:

Over the coming year the following will be pursued:

1. Continuing studies on the fine structure of the α and β chains of the receptor, particularly preparative isolation of the α chain domains if possible without the use of denaturants.

2. Continued efforts to understand the nature of the $\alpha:\beta$ interaction

3. Preparative isolation of the β -chain in order to begin fine structure analysis of it as well as to make antibodies to it.

4. Continued studies on reincorporated receptors and use of such liposomes to perform structural and functional studies.

5. Experiments to demonstrate directly that fragments of irradiated IgE or receptor molecules retain binding activity.

6. Continued study of digestion of IgE-α subunit complexes both to study the nature of the IgE receptor interaction and to see if undenatured IgE-free receptors can be prepared in this way.

Publications

1. Holowka, D., Hartmann, H., Kanellopoulos, J. and Metzger, H: Association of the receptor for immunoglobulin E with an endogenous polypeptide on rat basophilic leukemia cells. J. Receptor Res. 1: 41-68, 1980. (previously listed as "In press")

2. Fewtrell, C. and Metzger, H: Larger oligomers of IgE are more effective than dimers in stimulating rat basophilic leukemia cells. J. Immunol. 125: 701-710, 1980. (previously listed as "In press")

3. Metzger, H., Holowka, D., Hartmann, H. and Fewtrell, C: Molecular mechanisms of antibody action: use of cross-linking reagents. In Molecules, Cells and Parasites in Immunology eds. C. Larralde, K. Wilms, L. Ortiz-Ortiz, M. Sela) Academic Press, N.Y., pp. 1-11, 1980.

4. Fewtrell, C. and Metzger, H: The role of aggregation in the function of the IgE Fc receptors. J. Retic. Endo. Soc. 28: 35S-42S, 1980. (previously listed as "In press")

5. Kanellopoulos, J., Liu, T.Y., Poy, G. and Metzger, H: Composition and subunit structure of the cell receptor for immunoglobulin E. J. Biol. Chem. 255: 9060-9066, 1980. (previously listed as "In press").

6. Metzger, H., Kanellopoulos, J., Holowka, D., Goetze, A. and Fewtrell, C: Structural analyses of the receptor for IgE on rat basophilic leukemia cells. Biochem. of Acute Allergic Reactions, 1981. (previously reported as "In press")

7. Fewtrell, C. and Metzger, H: Stimulus-secretion coupling in rat basophilic leukemia cells. Biochem. of Acute Allergic Reactions, 1981 (previously listed as "In press").

8. Fewtrell, C., Lagunoff, D. and Metzger, H: Secretion from rat basophilic leukemia cells induced by calcium ionophores: Effect of pH and metabolic inhibitors. Bioch. Biophys. Acta. 644: 363-368, 1981.

9. Holowka, D., Gitler, C., Bercovici, T. and Metzger, H: Reaction of 5-iodonaphthyl-1-nitrene with the receptor for IgE on normal and tumor mast cells. Nature 289: 806-808, 1981.

10. Metzger, H., Holowka, D., Kanellopoulos, J. and Fewtrell, C: Antigen-Induced Antibody Mediated Transmembrane Signalling: The IgE Mast Cell System. Proceed. XI Cong. Europ. Allergol. & Clin. Immunol. (Ludwig and Steffen eds.), 1981. Elsevier/N. Holland, Amsterdam, pp. 331-338.

11. Holowka, D. and Metzger, H: Further Characterization of the β component of the receptor for IgE. Molec. Immunol. In press, 1981.

12. Kagey-Sobotka, A., Dembo, M., Goldstein, B., Metzger, H. and Lichtenstein, L.M: Qualitative characteristics of histamine release from human basophils by covalently crosslinked IgE. J. Immunol. In press, 1981.

13. Goetze, A., Kanellopoulos, Rice, D. and Metzger, H: Enzymatic cleavage products of the alpha subunit of the receptor for IgE. Biochemistry. In press, 1981.

14. Fewtrell, C., Kempner, E., Poy, G. and Metzger, H: Unexpected findings from target analysis of immunoglobulin E and its receptor. Biochemistry. In press, 1981.

15. Metzger, H., Goetze, A., Kanellopoulos, J., Holowka, D. and Fewtrell, C: Structure of the high affinity mast cell receptor for IgE. Fed. Proc. In press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41029-05 ARB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of Computerized Clinical Database for Systemic Lupus Erythematosus (SLE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.H. Klippel	Senior Investigator	ARB NIAMDD
OTHER:	J.L. Decker	Chief	ARB NIAMDD
	E.K. Harris	Chief	LAS DCRT
	G. Shakarji		DMB DCRT

PROJECT INACTIVE

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41035-04 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Lymphocyte Depletion in the Treatment of Rheumatoid Arthritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.L. Decker	Chief	ARB NIAMDD
	J. Karsh	Guest Worker	CIB NIDR
OTHER:	J.H. Klippel	Senior Investigator	ARB NIAMDD
	A.B. Deisseroth	Chief	POB NCI
	D.G. Wright	Senior Investigator	POB NCI
	W. Flye	Senior Investigator	SURG NCI

PROJECT TERMINATED

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41036-04 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Epidemiology of Canine and Human Systemic Lupus Erythematosus (SLE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. H. Klippel	Senior Investigator	ARB NIAMDD
OTHER:	J.L. Decker	Chief	ARB NIAMDD
	A.D. Steinberg	Senior Investigator	ARB NIAMDD

PROJECT TERMINATED

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41037-04 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Genetic Factors in Systemic Lupus Erythematosus (SLE): Study of the Major Histocompatibility Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.H. Klippel	Senior Investigator	ARB NIADDK
	D.L. Mann	Senior Investigator	IB NCI
OTHER:	J.L. Decker	Chief	ARB NIADDK
	A.D. Steinberg	Senior Investigator	ARB NIADDK

PROJECT TERMINATED

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41038-04 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Renal biopsy pathology in systemic lupus erythematosus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.E. Balow	Senior Investigator	ARB/NIADDK

COOPERATING UNITS (if any)
Armed Forces Institute of Pathology, Washington, D.C.
(T.A. Antonovych, M.D.)

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detailed analysis of renal biopsy pathology has been undertaken in patients with systemic lupus erythematosus who are receiving various therapeutic regimens of immunosuppressive agents. Biopsies will be scored for various indices of pathological activity as well as for patterns of deposition of immune complexes by immunofluorescence and electron microscopy; these morphologic parameters are to be related to renal function data in order to ascertain whether more accurate prognostic indices can be developed and whether more sensitive measures of therapeutic response can be identified.

PHS-6040

Project Description:

Objectives:

The classification of renal biopsy pathology in systemic lupus erythematosus (SLE) into general categories based mainly on the extent of cellular proliferation of glomerular cells as assessed by light microscopy has been a relatively poor predictor of prognosis in treated or untreated patients. Following immunologically mediated renal injury, renal function may improve by either regression of inflammatory changes in involved glomeruli or by compensatory increases of function in less involved glomeruli. The latter mechanism may explain why renal function is generally poor measure of cumulative renal damage until late in the chronic phases of nephritis when renal reserve capacity has been exhausted. Direct histological examination of renal biopsy material should provide a means to identify the extent of disease irrespective of the degree of renal functional compensation. The present study will examine the morphologic characteristics of SLE nephritis over time in patients being studied in ongoing therapeutic protocols. Biopsies will be scored for specific pathologic parameters which will be used as semi-quantitative indices of disease activity or chronicity. Repeat renal biopsy will be performed for evaluation of the same parameters at a specific time period, i.e. two years, regardless of the apparent clinical response of the patient to ascertain whether more accurate prognostic indicators can be identified and whether more sensitive measures of therapeutic response can be identified.

Methods Employed:

Percutaneous renal biopsy is performed under direct fluoroscopic visualization. Tissue samples are processed for routine light, immunofluorescence and electron microscopy. Sections are blinded and scored by both a nephropathologist and a clinical nephrologist on a checklist of specific morphological parameters.

Major Findings:

Clinical and routine laboratory studies may not identify patients with morphologically active disease as determined by renal biopsy. Moreover, in those patients with clinically active renal disease, no specific parameter has been found to be predictive of the distribution nor the severity of pathologic lesions. We have found that patients with renal biopsies having a classification of diffuse proliferative glomerulonephritis have a broad spectrum of severity as judged by the type and number of active lesions either within glomeruli or in the interstitium. In addition the significance of sclerosing lesions, which may be concomitantly present with proliferation, appears to be an important prognostic factor which has not received adequate consideration in most series. Studies of serial renal biopsies using the semi-quantitative histological scoring system have been undertaken in patients who have entered a chronic stable phase of lupus nephritis. Preliminary results suggest that renal histologic changes are more severe in patients receiving corticosteroids alone compared to those receiving cytotoxic agents in addition. Analysis of larger numbers of patients is actively underway.

Significance to Bio-Medical Research and the Program of the Institute:

An improved system for evaluation and classification of renal biopsy pathology would provide the following advantages:

1. A clearer description of the types of pathology in SLE nephritis.
2. The ability to select more patients with more nearly homogenous types of nephritis for study of therapeutic responses.
3. Improved ability to identify accurate prognostic indicators of subsequent renal function during long-term therapeutic studies.
4. Provide an early index more sensitive than renal function as a measure of outcome in immunosuppressive drug trials in SLE nephritis.
5. Improved bases of indications for initiating and terminating therapy for SLE nephritis.

Proposed Course:

An ongoing study of patients who will be asked permission for repeat renal biopsy every two years is planned. Patients will be studied as they enter new therapeutic protocols as well as those followed in previous treatment programs.

Publications:

1. Balow, J.E: Therapeutic trials in lupus nephritis. Problems related to renal histology, monitoring of therapy and measures of outcome. Nephron (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41039-04 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Regulation of cell mediated immune responses in normals and patients receiving immunosuppressive therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.E. Balow	Senior Investigator	ARB NIADDK

PROJECT TERMINATED

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 41040-09 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of various cytotoxic drug programs in diffuse lupus nephritis (PACIV)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.D. Steinberg	Senior Investigator	ARB NIADDK
	J.L. Decker	Chief, Arthritis and Rheumatism Branch	ARB NIADDK
OTHER:	P.H. Plotz	Senior Investigator	ARB NIADDK
	J.H. Klippel	Senior Investigator	ARB NIADDK
	J.E. Balow	Senior Investigator	ARB NIADDK
	R. Wilder	Senior Investigator	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with the diffuse forms of systemic lupus erythematosus nephritis were invited to enter a therapeutic trial. The three programs were corticosteroids only; corticosteroids with cyclophosphamide and azathioprine, both in low doses; and corticosteroids with intermittent intravenous cyclophosphamide.

Regular follow-up of the patients admitted continues.

Project Description:

Objectives:

The Arthritis and Rheumatism Branch has a major interest in the management of the nephritis of systemic lupus erythematosus (SLE). Renal failure is the most common cause of death in the disease. The renal damage is produced by immune complex deposition in the glomeruli. It has been suggested that drugs capable of altering the antibody response, "immunosuppressives," would reduce the formation of antibody and thus of antigen-antibody complexes. The objective of this study is to assess the efficacy and toxicity of various immunosuppressive drug programs in SLE nephritis.

Methods Employed:

Patients with systemic lupus erythematosus and active nephritis are invited to enter the trial. All patients are to be on prednisone, and one group will receive no additional medications. The other two groups receive in addition: oral cyclophosphamide up to 1 mg/kg/day and azathioprine up to 1 mg/kg/day; or every 3 months intravenous infusions of cyclophosphamide up to 1 gm/M^2. All patients are repeatedly reevaluated with a battery of renal function tests, serological studies, urinalyses and, after two years in the trial, by renal biopsy when possible. It is proposed to continue the treatment programs of admitted patients indefinately. Treatment failures can be withdrawn according to protocol.

Major Findings:

Forty-one patients have been followed at least one year [13 on prednisone only (PO); 16 on cyclophosphamide plus azathioprine (CA), and 12 on IV cyclophosphamide (IC)]. Four patients have died -- none from renal failure or an abvious drug toxicity. Two patients stopped CA because of a desire to become pregnant and 2 stopped CA for drug side effects. Decline in renal function (serum creatine by 25%) was observed in 4 patients on PO, 2 patients on CA, and one patient on IC. Proteinuria and hematuria remained stable or improved in almost all patients.

Non-renal SLE flares occurred more commonly ($x^2=6.1$, $p<0.05$) in PO patients (13 in 39.2 observation-years) than in the other two groups (6 in 51.3 years with CA + 5 in 39.4 years with IC = 11 in 90.7 years).

Complications have included marked increase in aseptic necrosis in PO patients and increased herpes zoster in the other two groups.

Although decline in renal function has occurred somewhat more commonly in PO patients (4/12 = 33%) than in the two cytotoxic groups (3/27 = 11%), this difference has not reached statistical significance. Nevertheless, this encouraging trend coupled with fewer non-renal SLE flares in the IC and CA groups plus the increase in aseptic necrosis of bone in the PO group justifies continuation of the study.

Significance to Bio-Medical Research and the Program of the Institute:

These studies are relied upon by physicians concerned with SLE all over the world. We have been one of very few units able to speak from the hard data base of controlled studies. The net effect has been a great reduction in unwarranted enthusiasm for these agents. These studies certainly show that other modes of alleviating SLE renal disease are needed.

Proposed Course:

We will continue to follow these patients on their assigned programs. We have devised an accelerated program of intravenous cyclophosphamide versus intravenous corticosteroids to treat accelerated or histologically active renal disease in patients with SLE.

Publications:

1. Decker, J.L.: Causes of Death in Systemic Lupus Erythematosus (In Portuguese). Revista da Sociedade Brasileira de Rheumatologia. (In press).

2. Steinberg, A.D., and Steinberg, S.C.: "Autoimmune disease and the clinical consequences of prolonged immunosuppression." In, Inadvertent Modification of the Immune Response. The Effects of Foods, Durgs, and Environmental Contaminants. Proc. of Fourth FDA Science Symposium, Aug. 1978. Asher, I.M. (Ed.) HHS Publication No. (FDA) 80-1074, 1980, p. 39-51.

3. Steinberg, A.D.: "Immunoregulatory drugs." Chapter 55. In Kelley, W.N., Harris, E.D., Ruddy, S., and Sledge, C.B. (Eds.): Textbook of Rheumatology, Philadelphia, W.B. Saunders Co., 1980, pp. 841-861.

4. Steinberg, A.D.: "Management of Systemic lupus erythematosus." Chapter 70. In Kelley, W.N., Harris, E.D., Rudd, S., and Sledge, C.B. (Eds.): Textbook of Rheumatology. Philadelphia, W.B. Saunders Co., 1980, pp. 1133-1150.

5. Steinberg, A.D.: Modulation of a complex immune system. Ann. Neurology 9 suppl:117-124, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41041-03 ARB

PERIOD COVERED
October 1, 1980 to September 30, 19881

TITLE OF PROJECT (80 characters or less)

Aspirin and ibuprofen in SLE

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Plotz	Senior Investigator	ARB NIADDK
OTHER:	D. Magilavy	Clinical Associate	ARB NIADDK
	D. Finbloom	Clinical Associate	ARB NIADDK
	J.L. Decker	Chief, Arthritis and Rheumatism Br.	ARB NIADDK
	J. Karsh	Guest Worker	CIB NIDR

PROJECT TERMINATED

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41042-03 ARB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Intravenous Cyclophosphamide in the Treatment of Rheumatoid Arthritis (RA)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.H. Klippel	Senior Investigator	ARB NIAMDD
OTHER:	J.L. Decker	Chief	ARB NIAMDD
	P.H. Plotz	Senior Investigator	ARB NIAMDD
	N.L. Gerber	Chief	REHAB CC

PROJECT TERMINATED

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41043-03 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Immunization of Patients with Systemic Lupus Erythematosus (SLE) with Pneumococcal Polysaccharide Vaccine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.H. Klippel	Senior Investigator	ARB NIAMDD
OTHER:	J.L. Decker	Chief	ARB NIAMDD

PROJECT TERMINATED

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41044-03 ARB

PERIOD COVERED

October 1, 1980 to September 31, 1981

TITLE OF PROJECT (80 characters or less)

Lymphocyte Depletion vs Plasma Removal in the Treatment of Rheumatoid Arthritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.L. Decker	Chief	ARB NIAMDD
OTHER:	J.H. Klippel	Senior Investigator	ARB NIAMDD
	J. Karsh	Guest Worker	CIB NIDR
	D.G. Wright	Senior Investigator	POB NCI
	A.B. Deisseroth	Chief	POB NCI
	W. Flye	Senior Investigator	SURG NCI

PROJECT TERMINATED

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41048-02 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Study of Genetic and Pathophysiologic Features in the Rat of Experimental Erosive Synovitis Induced by Bacterial Cell Walls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.L. Wilder	Clinical Associate	ARB NIADDK
Other:	G.B. Calandra	Senior Investigator	LSD NIAID
	C.T. Hansen	Chief	VR RR
	L.M. Wahl	Senior Investigator	LMI NIDR
	S.M. Wahl	Senior Investigator	LMI NIDR
	J.B. Allen	Biochemist	ARB NIADDK

COOPERATING UNITS (if any)
Laboratory of Streptococcal Diseases, NIAID; Veterinary Resources Branch, RR; Laboratory of Microbial Immunology, NIDR

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.75	PROFESSIONAL: 0.75	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Erosive synovitis can be induced experimentally in rats by intraperitoneal injection of bacterial cell walls from group A streptococci. Genetic factors determine susceptibility to development of disease. Susceptible inbred rat strains develop marked mononuclear cell inflammation, most evident in peripheral joints. Resistant inbred strains show little evidence of pathology. Susceptibility is a dominant trait, but is determined by multiple genes. Immunologic characterization has revealed marked differences in response to cell walls between susceptible and resistant strains.

Project Description:

Objectives:

Published data show that experimental erosive synovitis can be elicited in rats by intraperitoneal injection of cell walls from certain groups of streptococci. Studies in progress have the following goals:

1. To define the variability in incidence, type and severity of the experimental arthritis.
2. To define the properties of bacteria that facilitate development of arthritis.
3. To characterize the host genetic factors which determine susceptibility to arthritis.
4. To better define pathophysiologic processes by characterizing differences between high and low responder inbred rat strains.

Methods Employed:

1. Bacterial cell walls, free of membrane, are isolated from Streptoccoccus pyogenes, Group A, solubilized by sonication, and used to induce arthritis in various outbred and inbred rat strains.
2. Rats, both inbred and outbred, are injected intraperitoneally with the cell wall preparations.
3. The rats are observed frequently to document the time of onset severity and characteristics of the arthritis and other clinically evident pathology. Additional assessment is made by radiography and histology.
4. Selected inbred strains are used in breeding studies to determine the importance of selected genetic determinants such as the major histocompatibility (MHC) and Ig allotype complexes.
5. Selected strains are analyzed in terms of macrophage and lymphocyte function

Major Findings:

1. More than 50 outbred sprague-dawley rats have been studied. About 30-40% develop a distal destructive arthritis with variable severity. These results suggest genetic variability influences the development of disease.
2. Sixteen inbred rat strains have been studied. A genetic predisposition to disease has been unequivocally demonstrated. Several strains are 100% susceptible; several others are markedly resistant.
3. Susceptibility is a dominant genetic trait but is determined by more than three genetic loci including non MHC determinants.

4. The characteristic pathologic lesion is a chronic mononuclear cell infiltration consisting of macrophages and lymphocytes in addition to marked synovial cell proliferation.

5. Development of arthritis appears to depend on the persistence of cell wall materials for prolonged periods of time in the affected tissue.

6. Following injection, resistant strains develop marked macrophage mediated suppression of lymphoproliferative responses to a variety of stimuli. This presists for more than 16 weeks. In contrast, susceptible strains develop transient suppression peaking at about 6 weeks, but this is followed by a macrophage (and ? T cell) mediated enhancement of lymphoproliferative responses to various stimuli.

7. Macrophages from resistant strains respond to cell walls with prostaglandin E_1 release of a higher magnitude and wider dose response range than do susceptible strains.

Significance to Bio-Medical Research and Program of the Institute:

Peptidoglycan containing bacterial cell walls have received increasing attention in recent years as possible pathogenetic agents in some cases of chronic synovitis. This animal model provides a potentially powerful tool to incisively explore the role of genetic and immunologic parameters in the development of chronic synovitis..

Proposed Course:

The genetic and pathophysiologic characterization of this experimental model is continuing.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41049-02 ARB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The comparative efficacy and safety of azathioprine and D-penicillamine for the treatment of rheumatoid arthritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. H. Plotz	Senior Investigator	ARB NIADDK
OTHER:	J. L. Decker	Chief, Arthritis & Rheum. Br.	ARB NIADDK
	J.H. Klippel	Senior Investigator	ARB NIADDK
	R. Wilder	Clinical Associate	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, patients with rheumatoid arthritis unresponsive or intolerant to gold therapy will be randomized in a double-blind trial to receive either azathioprine or d-penicillamine. The trial will compare the efficacy and toxicity of these during a 24 week trial and an extended follow-up observation period. The Arthritis and Rheumatism Branch is one of eleven clinics carrying out this protocol under the direction of the Center for Cooperative Studies in the Rheumatic Diseases.

Project description

Objectives

1. To compare, in patients with rheumatoid arthritis, the effectiveness of azathioprine and d-penicillamine.

2. To compare severity and incidence of side effects and complications among the two treatment groups.

Methods Employed

In this cooperative trial, a total of 200 patients with adult onset rheumatoid arthritis will be evaluated in a double-blind, parallel study for a duration of 24 weeks. Patients will be randomized to either azathioprine or d-penicillamine and seen in the out-patient clinic every 2 weeks during this study. Clinical efficacy of the drugs will be evaluated and compared by periodic assessments of the articular inflammation and x-ray changes.

Major Findings

None at this time

Significance to Bio-Medical Research and Program of the Institute

The careful study of the comparative efficacy and safety of these two long-acting anti-rheumatic drugs will be of great assistance in the medical management of patients with rheumatoid arthritis.

Proposed Course

To date a total of seven patients from the Arthritis and Rheumatism Branch clinic population have enrolled in the trial. These patients will continue to be followed. In addition, new patients for the trial are being actively recruited.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41050-02 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Comparative efficacy and safety of myochrysine and auranofin in the treatment of rheumatoid arthritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Plotz	Senior Investigator	ARB NIADDK
OTHER:	J.L. Decker	Chief, Arthritis & Rheum. Br.	ARB NIADDK
	J.H. Klippel	Senior Investigator	ARB NIADDK
	R. Wilder	Clinical Associate	ARB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gold has been known to have a beneficial effect on rheumatoid arthritis for many decades. In this study, a new oral gold preparation (auranofin) will be compared to a placebo and to intramuscular gold in a double-blind trial involving 11 clinics cooperating under the aegis of the Center for Cooperating Studies in the Rheumatic Diseases.

Project description

Objectives

1. To compare in patients with definite or classical active progressive rheumatoid arthritis the effectiveness of auranofin, gold sodium thiomalate, and placebo.

2. To compare incidence and severity of side effects and complications amongst the three treatment groups.

Methods Employed

The study in a 21 week out-patient double-blind parallel trial of auranofin, gold sodium thiomalate, or placebo with a one year follow-up of patients receiving auranofin. Patients are to be seen in the clinic weekly to receive either intramuscular gold thiomalate and daily placebo tablets, placebo injections and daily auranofin tablets, or placebo injection and placebo tablets. Clinical efficacy will be evaluated by periodic assessment of articular inflammation and x-ray changes amongst the 3 groups. After 21 weeks in the double-blind trial, all patients will be eligible for a one-year open trial of auranofin.

Major Findings

None at this time

Significance to Bio-Medical Research and Program of the Institution

To find a form of gold of comparable efficacy and safety to standard intramuscular gold which can be taken by mouth would be a useful advance in the management of rheumatoid arthritis.

Proposed Course

The enrollment phase of this cooperative trial has been completed with a total of 220 patients entered. Three patients from our clinic population have entered this trial. During the coming year, these patients will continue to be followed and provided the opportunity to enter the open phase at the completion of 21 weeks.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41051-02 ARB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Natural History and in situ organization of the receptors for IgE.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	C. Isersky-Carter, Senior investigator	ARB, NIADDK
Others	H. Metzger, Chief, Section on Chemical Immunology	ARB, NIADDK

COOPERATING UNITS (if any)
R. Siraganian LMI, NIDR., L. Basciano, D. Segal I.B., T. Triche L.P., NCI

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the natural history and in situ organization of the receptor for IgE on mast cells and basophils. Various sublines of rat basophilic leukemia (RBL) cells and plasma membrane vesicles prepared from them are used. Purified IgE myeloma, antibodies to IgE and to the receptor are also used. These studies will help clarify the mode of function of the receptor, the fate of the receptor-bound monomeric and cross-linked IgE and the turnover of the free receptor.

Project Description

Objectives

The various rat basophilic leukemia cell lines as well as purified labeled IgE myeloma and hybridoma provide a unique model system with which one can follow the fate of a protein that binds specifically and tightly to a surface receptor. The main objectives are to investigate the conditions under which the surface bound IgE is internalized and the effect of internalization on the turnover of the receptor. These cell lines also provide a source of plasma membrane vesicles carrying purified radiolabeled marker for the outer leaflet of the plasma membrane, namely IgE. The main objective here is to elucidate the manner in which the receptor is organized in the membrane, and thus learn about the mechanism involved in transmitance of signals across the cell membrane.

Major findings

A. Internalization of oligomers of IgE and its effect on receptor turnover.

The questions we addressed were 1) the size of the signal required for internalization; 2) the role if any, of IgG Fc receptor in the internalization of the IgE oligomers; 3) the fate of the various internalized oligomers of IgE and IgG (i.e. disposition, intracellular translocation, shuttling); 4) the fate of the ligand and the receptor in the process of internalization. Though dimers were shown to be less effective in initiating exocytosis of histamine than trimers and the latter were less effective than heavier oligomers, we found that dimers, trimers and heavier oligomers of IgE were internalized at similar rates ($t_{\frac{1}{2}} \sim \frac{1}{2}$-1 hr.) and to similar extents. Internalization of dimers and trimers occurred equally well in histamine releasing and non-releasing sublines. This indicates that exocytosis in these cell lines cannot be directly and simply related to the internalization of the bulk of the surface bound ligand. Importantly, both cell lines were capable of distinguishing between the binary complex of receptor and IgE and the ternary complex in which two receptors have presumably been linked by one dimeric molecule of IgE. Thus minimal receptor aggregation brought forth efficient internalization. Cell surface bound oligomers of IgG were also internalized. Though IgG receptors have been shown to bind IgE oligomers the internalization of either oligomer apparently was unaffected by excess of the heterologous monomer. We therefore, conclude that the internalization is driven by the specific receptor. Short term (½-6 hrs.) studies show that the process of internalization of radiolabeled ligand is temporally associated with appearance of TCA non-precipitable label in the culture medium. Therefore some ligand degradation occurs concurrently with internalization. During the same time no significant reexpression of unoccupied receptors on the cell surface or in cell extracts could be demonstrated. This indicates that the receptor remains inaccessible and is neither rapidly recycled nor replaced during and immediately following ligand internalization.

B. Assessment of the utility of RBL membrane-vesicles in determining whether the IgE receptor is a transmembrane protein.

We developed a reproducible procedure for the preparation of membrane vesicles of a predictable quality and yield from RBL cells, partially saturated with radiolabeled IgE, the latter served as a plasma membrane marker. Assessment of solubilized vesicles indicated that during membrane preparation free receptors and receptor-IgE complexes were well preserved and no major losses of other radiolabeled surface proteins occurred. Electron microsopic examination revealed that these preparations were rich in smooth vesicles and contained few contaminating sub-cellular organelles. All of the vesicle preparations tested failed to bind the expected amount of IgE and contained a high proportion (40-80%) of vesicles that failed to bind to affinity columns carrying anti-IgE on concanovalin A. Solubilization in non-ionic detergent resulted in recovery of the expected capacity of free receptor to bind IgE and of the IgE-receptor complexes to bind to anti-IgE affinity columns. These data could indicate the presence of reversed vesicles as well as the potential usefulness of affinity columns in sorting vesicles according to their orientation. However further studies showed that vesicle associated IgE bound proportionately more soluble IgG-anti-IgE than was expected from studies on whole cells, additionally processing on affinity columns did not affect the reactivity with soluble anti-IgE or IgE.

To reconcile all of these findings one would have to assume that A) the preparations contained a high proportion of relatively permeable 'reversed' vesicles and B) that in the process of making the vesicles the free receptor was rendered physically less accessible to IgE, while the receptor bound IgE was rendered more accessible to soluble anti-IgE. The vesicles were, indeed, found to be permeable by dextran gradients and electronmicroscopy. Studies using an E.M. identifiable outer membrane marker (IgE-Ferritin) were carried out to verify the presence of reversed vesicles. However results obtained so far did not allow a clear cut conclusion with respect to vesicle orientation.

Inspite of the cited uncertainty we believed that the inner facet of the membrane was rendered accessible. We therefore proceeded with labeling and enzyme digestion studies, aimed at detecting an intramembranous portion of the receptor. We found that vesicle formation rendered some membrane proteins less, and some more accessible to labeling, however the IgE-occupied receptor on whole cells and membrane vesicles were equally unavailable to lactoperoxidase catalyzed iodination. Treatment of vesicles with papain and trypsin had no effect on the ability to bind IgE or on the apparent molecular weight of the α chain of the receptor as determined on SDS PAGE.

Though these observations could suggest that the IgE receptor is not a transmembrane protein, three caveats must be mentioned. Tyrosin phenylalamin and histidine free portions of the receptor, which traverse the membrane, would not be detected by lactoperoxidase catalyzed iodination. Secondly only two proteases were used, it is possible that the potential cytoplasmic portion of the receptor is not digestable by either. Thirdly, it is difficult to demonstrate rigorously that the inner facet of the membrane was available to the enzymes and the second label.

C. Assessment of the anti-genic relationship between IgE and IgG Fc receptors on mouse mastocytoma cells.

Rat basophilic leukemia cells and mouse mastocytoma cells have receptors for the Fc portion of IgE and IgG. The IgE receptors bind IgE exclusively while the IgG receptor binds IgE oligomers as well, thus suggesting that the two receptors could be related; if so antiserum from rabbits which were hyperimmunized with either receptor might be expected to interact with both. To study this we used non-ionic detergent extracts of surface labeled mouse mastocytoma cells, rabbit antiserum to IgE Fc receptors of RBL cells, antiserum to IgG Fc receptors of mouse peritoneal macrophages and IgE together with affinity purified anti-IgE antibodies. We found that antiserum to rat IgE receptor reacted mainly with one membrane component of mouse mastocytoma cells, the apparent molecular weight of this receptor on SDS PAGE was ~ 60,000, pretreatment of extracts with IgE and anti-IgE eliminated all reactivity with this antiserum. Antiserum to mouse IgG Fc receptor reacted mainly with a component with an apparent M.W. of ~ 68,000. This antiserum failed to react with extracts from surface labeled RBL cells, though these cells were shown to have an IgG receptor. Pretreatment of extracts from surface labeled IgE saturated mouse cells with anti-IgE alone or anti-IgE followed by antiserum to rat IgE receptor did not eliminate reactivity with the antiserum to mouse IgG receptors. Pretreatment with anti-IgG receptor alone did not eliminate reactivity with antiserum to the IgE receptor. Our studies confirm that IgE and IgG receptors are two separate anti-genically distinguishable membrane components; so far we have failed to find major antigenic similarities between them.

Significance to Bio-Medical Research and the Program of the Institute:

Basophils, mast cells and IgE are important components of the allergic and inflammatory reactions. They provide a useful model system for studying the organization of receptors in membranes as well as the fate of cell bound Ligands (IgE), the condition that leads to their endocytosis and the latters effect on surface expression and turnover of receptors. Knowledge of these may aid in developing methods aimed at modulating the allergic and inflammatory reactions.

Proposed course:

1. Continue to study the effect of Ligand presence and internalization on turnover of the receptors; verify the fate of receptors during and following the event of Ligand internalization; study the intracellular shuttling and disposition of the internalized IgE.

2. Continue to study the in situ organization of the chain of the receptor for IgE and attempt to define whether the chain is a transmembrane protein.

Publications

1. Barsumian E.L., Isersky, C., Petrino, M. and Siraganian, R: IgE-induced histamine release from rat basophilic leukemia cell lines: Isolation of releasing and non-releasing clones. Eur. J. Immunol. European Journal of Immunology 11: 317-323, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AM41052-02 ARB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Disorders of immune regulation in patients with systemic lupus erythematosus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Balow	Senior Investigator	ARB NIADDK
Other:	G. C. Tsokos	Visiting Fellow	ARB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with systemic lupus erythematosus have been found to have disturbances of cell-mediated immune responses. Alterations of B and T lymphocytes as well as monocyte function have been observed both in patients with SLE and certain of their relatives. The major goal of these studies is to further characterize the types of immunological disturbances in SLE and to elucidate the mechanisms which lead to alterations of immune regulation and are responsible for the pathogenesis of this disease.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives

SLE is considered to be caused by disturbances in immune regulation. Although several disorders of the T and B lymphocyte functions have been noted in patients with SLE, certain of these abnormalities: 1) are absent in some affected SLE patients, 2) continue to be present after patients enter remission of SLE, and 3) have also been observed in non-affected relatives of SLE patients. Thus, the questions regarding which abnormalities are necessary and/or sufficient for the pathogenesis of SLE remain unclear. The present studies seek to elucidate certain of the mechanisms causing abnormal cell-mediated immunological dysfunction in order to further understand the pathogenesis and to consider novel approaches to therapy of SLE.

Methods Employed:

1. B lymphocyte functions will be studied by enumeration of immunoglobulin secreting cells in reverse hemolytic plaque assays. Various subpopulations of lymphoid cells from SLE patients will be tested in combination in order to elucidate the types of cells with either excessive or deficient immunoregulatory function.

2. T lymphocyte functions will be studied in assays of cell-mediated cytotoxicity, mixed leucocyte reaction and lymphocyte proliferation in response to various in vitro mitogenic stimuli. The role of autoantibodies directed against selected lymphocyte subpopulations, as well as the role of circulating immune complexes, in causing disturbances of immune regulation will be studied.

3. Studies of cellular interactions involving T and B lymphocytes and monocytes will be undertaken to further determine the state of helper and/or suppressor cell subsets in patients with SLE as compared to those of normal subjects.

Major Findings:

Patients with SLE have been found to have elevated numbers of spontaneously activated immunoglobulin secreting cells in their peripheral leucocytes. Patients with the most clinically active disease have the highest proportion of activated B lymphocytes which, however, do not respond normally to stimulation with polyclonal activators, such as pokeweed mitogen. However, a radiation-resistant accessory lymphoid cell from normal donors partially restores this defective response to B cell mitogens. These studies suggest that patients with SLE may lack a helper cell function necessary for normal B lymphocyte response, or that prior activation of B lymphocytes in vivo leads to refractoriness to mitogenic stimuli. Alternatively, the emergence of suppressor cell activity following in vivo B lymphocyte activation may lead to inhibition of immunoglobulin secretion.

Deficiency of T cell functions have been found in patients with SLE. Certain patients lack the ability to generate cytotoxic T lymphocytes to alloantigens, while nearly all patients exhibit a normal proliferative response to alloantigens in mixed leucocyte culture. Preliminary experiments suggest that the defect in ability to generate cytotoxic T lymphocytes may be caused by pre-activated suppressor cells in certain patients with SLE.

Significance to Bio-Medical Research and the Program of the Institute

The complex interacting and regulatory network of the normal immune response may reflect perturbations several cell levels distant from the primary disturbance. Clarification of the mechanisms causing each immunologic disturbance in SLE is necessary in order to ascertain their relative importance in the pathogenesis of SLE.

Proposed Course:

Continued studies of cell mediated lymphocyte functions of patients with SLE in various levels of disease activity in comparison to those of normal controls will be undertaken.

Publications:

1. Tsokos, G.C. and Balow, J.E: Cytotoxic responses to alloantigens in systemic lupus erythematosus. J. Clin. Immunol. (In press)
2. Tsokos, G.C. and Balow, J.E: Spontaneous and pokeweed mitogen-induced plaque forming cells in systemic lupus erythematosus. Clin. Immunol. Immunopathol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41053-02 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Effects of High-Dose Intravenous Methylprednisolone on the Course of Rheumatoid Arthritis (RA)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.L. Wilder	Clinical Associate	ARB NIAMDD
Other:	J.H. Klippel	Senior Investigator	ARB NIAMDD
	J.L. Decker	Chief	ARB NIAMDD
	A.S. Fauci	Senior Investigator	LCI NIAID
	P.H. Plotz	Senior Investigator	ARB NIAMDD
	N.L. Gerber	Chief	REHAB CC
	P.K. Navang	Senior Staff Fellow	CPRL/PDS CC

COOPERATING UNITS (if any)
None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Part one

Six patients with rheumatoid arthritis (RA) unresponsive to conventional therapies have been studied in an open pilot trial of short-term pulse methylprednisolone (MP). Following administration of 1 gram of MP intravenous on 3 consecutive days, dramatic changes in articular index were evident in the first 1-2 weeks. The effect persisted for a variable period of time - 2 weeks to more than 8 weeks. No major side effects were observed. No persistent changes in several immunological parameters were measured. The results provide a preliminary basis to design better therapeutic protocols

Part two

Oral administration of high dose pulse corticosteroid is potentially a more practical method of administering pulse therapy. Drug absorption and patient acceptance, however, of high dose orally administered steroids are not well documented. Thus, we studied plasma MP levels in 3 patients with RA after a single IV or oral administration. Preliminary results indicate that orally administered high dose MP is well absorbed (>60%.) and well tolerated, but therapeutic equivalence of IV versus orally administered MP is not certain.

Project Description:

Part I

Objectives:

Although corticosteroids are dramatically effective in diminishing inflammation, their administration to patients with rheumatoid arthritis on a prolonged daily basis is often associated with severe complications. Administration of steroids by intermittent high dose "pulses" is frequently effective in renal transplant rejection, whereas administration by conventional regimens are usually ineffective. This mode of administration has been generally safe. Because we suspect that transplant rejection and rheumatoid synovitis may have some cell-mediated inflammatory mechanisms in common, an open pilot study of high dose pulse methylprednisolone (MP) therapy was initiated. Objectives of the study were:

1. To determine if short-term pulsed intravenous (IV) MP could induce significant improvement of subjective and objective signs of inflammatory arthritis in patients with rheumatoid arthritis.
2. To determine the nature of an duration of any beneficial effects.
3. To document incidence, type, and severity of reactions of this mode of treatment.

Methods Employed:

1. Patients admitted to the study met criteria for definite or classic RA, had persistently active disease, and had failed to adequately respond to non-steroidal anti-inflammatory agents and at least 2 other major anti-rheumatic drugs (gold, antimalarials, penicillamine or cytotoxic) drugs. Cytotoxic drugs, if previously tried, were discontinued 2 months prior to entering the study. Other drugs, including low dose corticosteroids, were maintained on a stable regimen for 6 months prior to pulse treatment.
2. The study consisted of 3 phases: a) pre-pulse MP evaluation (one month) to establish baseline parameters; b) pulse MP administration (5 days in hospital); single three-day course of IV MP, 1 gm/day; c) post-pulse MP evaluation (two months).
3. Each patient was assessed by a single examiner while in the study.
4. A variety of chemical, hematological and immunological parameters were assessed.

Major Findings:

1. All patients showed dramatic improvement measured by multiple parameters at one week. The improvement lasted for a variable period of time - 2 weeks (one patient) to more than 8 weeks (three patients).
2. No major side effects were observed. Minor side effects included transient insomnia, increased dreaming, flushing and a metallic taste.

3. No persistent changes were noted in chemical, hematological or immunological parameters studied, except for a decrease in erythrocyte sedimentation rate.

Part 2

Objectives:

Oral administration of high dose pulse corticosteroid is potentially a more practical method of administering pulse therapy; however, drug absorption and patient acceptance is not well documented. Thus, in this study our purpose was to measure plasma MP levels at various times after administration to document absorption and the kinetics of plasma clearance of the drug and to monitor patient acceptance.

Methods Employed:

1. Three patients with RA and ancillary criteria similar to those in Part One were studied.
2. Each patient was administered 1.0 gram of MP in three forms: a) intravenously over 30-60 min; b) orally administered capsules; and c) orally administered suspension in flavored liquid.
3. Plasma samples were collected at various intervals over a 24 hour period and plasma levels of drug measured by high pressure liquid chromatography.
4. Patient tolerance was a ascertained by observation and patient interview.

Major Findings:

Preliminary analysis of the results indicate that orally administered MP is well absorbed ($> 60\%$), has a $T_{1/2}$ of $\sim$ 2-3 hours, and is well tolerated. Therapeutic equivalence to intravenously administered MP, however, is still not certain.

Significance to Bio-Medical Research and Program of the Institute:

Therapy for severe rheumatoid arthritis remains controversial and often inadequate. The results of these pilot studies support this view that pulse adminstration of corticosteroids in high doses may have a role in patient management, particularly as an intermittent adjunct to conventional antirheumatic therapy. Furthermore, the data would suggest that oral administration may be a more practical route of administration for outpatient intermittent therapy and warrants further study.

Proposed Course:

Further studies are being discussed.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41054-01 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Effect of a Brief Course of Lymphapheresis in the Management and Investigation of Rheumatoid Arthritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Wei	Clinical Associate	ARB NIADDK
OTHER:	R.L. Wilder	Clinical Associate	ARB NIADDK
	J.L. Decker	Chief	ARB NIADDK
	L. Wahl	Senior Investigator	LMI NIDR
	S. Wahl	Senior Investigator	LMI NIDR
	B. Stadler	Senior Investigator	LMI NIDR
	C. Yarboro	Clinical Nurse	ARB NIADDK
	I. Scher	Senior Investigator	NMRI NNMC
	I. Catona	Senior Investigator	NMRI NNMC
	J.B. Allen	Biochemist	ARB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK,NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nine patients with Rheumatoid Arthritis, unresponsive to conventional therapy, have been studied in an open trial of a short course of lymphapheresis (six procedures over two to three weeks). Immunologic characteristics of the mononuclear cells removed do not correlate with initial disease activity. However, diminished mononuclear cell reactivity to various stimuli, in general, predicted a favorable response to therapy. Abnormalities tended to resolve with therapy. Nonresponders generally exhibited normal reactivity. Clinical improvement was quite variable in degree and frequently short term. These studies into potential mechanisms of disease in rheumatoid arthritis and provide new data with which to assess apheresis as a therapeutic modality.

Project Description:

Objectives:

Lymphocyte depletion resulting form repeated removal of leukocytes using a centrifugal flow cell separator (lymphapheresis) has been shown to have modest ameliorative effect on rheumatoid arthritis (RA) (Karsh et al, in press). Numerous questions, however, still remain unanswered about this therapeutic modality. In this study, our objectives are to:

(1) Determine if a short course of lymphapheresis (six procedures over 2-3 weeks) would produce a clinical effect similar to that observed with fifteen procedures over 5 weeks, as used in the above cited study.

(2) Characterize by a variety of methods the mononuclear cells removed by lymphapheresis, and to correlate thesd findings with disease activity.

Methods Employed:

(1) Patients admitted to the study met criteria for definite or classic RA, had active disease (Ritchie Camp articular index of Greater than 30 and had 15 or more swollen or tender joints), had failed to adequately respond to non-steroidal anti-inflammatory agents and at least 2 other major anti-rheumatic drugs (gold, anti-malarials, penicillamine or cytotoxics). Cytotoxic drugs were stopped at least one month before entering the study. Other drugs, including low dose steroids were maintained on a stable regimen.

(2) The patients received six procedures over a two to three week period and then were followed for up to six months.

(4) Extensive characterization of the mononuclear cells removed at each procedure has been attempted.

Major Findings:

(1) Nine patients have been studied. The clinical response to lymphapheresis has been variable. Three patients have had no change or distinct worsening. The other patients have demonstrated variable degrees of improvement which has lasted from as little as 1 week to more than 6 months.

(2) No correlotion between initial disease activity and the immunologic characteristic of the peripheral blood mononuclear cells has been discerned.

(3) The data also suggest that patients with diminished delayed type hypersensitivity reactions to skin test antigens and diminished lymphoproliferative responses to soluble antigens and mitogens respond best. Patients with normal or enhanced responses have generally failed to respond.

(4) Patients which have responded generally exhibit evidence of and of increased number of "activated" lymphocytes and monocytes in the peripheral blood when compaired with normal individuals and patients who have not improved with lymphapheresis.

(5) Lymphoproliferative defects generally returned toward normal as therapy progressed in responders, but did not change in non-responders.

(6) No major side effects have been noted. One patient developed herpes zoster about two weeks after completing a course of lymphapheresis.

Significance to Bio-Medical Research and Program of the Institute:

Therapy for severe rheumatoid arthritis remains inadequate. Apheresis is an experimental technique which needs careful assessment. The studies in progress are providing new insights in desease mechanisms in this disease and provide additional data to assess the role of apheresis as a therapeutic modality.

Proposed Course:

The present studies are continuing.

Publications:

Karsh, J., Wright, D.G., Klippel, J.H., Decker, J.L., Deisseroth, A.B. and Flye, M.W: Arth and Rheum 22, 1055, 1979.

Karsh, J., Klippel, J.H., Plotz, P.H., Decker, J.L., Wright, D.G. and Flye, M.W: Lymphapheresis in rheumatoid arthritis: a randomized trial. Arthritis Rheum, In press.

Decker, J.L., Karsh, J. and Wilder, RL: Leukocytes and rheumatoid arthritis -treatment derived concepts. Compendium of XV International Congress of Rheumatology, Paris, 1981 - In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41055-01 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effect of Plasmapheresis on Systemic Lupus Erythematosus (SLE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Wei	Clinical Associate	ARB NIADDK
OTHER:			
	J.H. Klippel	Senior Investigator	ARB NIADDK
	J.E. Balow	Senior Investigator	ARB NIADDK
	T.H. Lawley	Senior Investigator	DB NCI
	R.P. Hall	Senior Investigator	DB NCI
	P.H. Plotz	Senior Investigator	ARB NIADDK
	A.D. Steinberg	Senior Investigator	ARB NIADDK
	Emilie Klima	Technician	ARB NIADDK
	Cheryl Yarboro	Nurse	CC
	William Campbell	Nurse	CC
	J.L. Decker	Chief	ARB NIADDK

COOPERATING UNITS (if any)

Dermatology Branch, NCI

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The therapeutic efficacy of short-term plasmapheresis in SLE will be assessed in a double-blind randomized trial. Patients will receive either 4 liter plasmapheresis or reinfusion plasmapheresis 3 times weekly for a period of 2 weeks. Clinical and laboratory assessments of lupus activity will be done with each pheresis and every week subsequent for 4 weeks. The protocol will be completed at the end of the 6th week or if the patient is withdrawn as a clinical therapeutic failure.

Project Description:

Objectives:

Plasmapheresis has been proposed as a possible alternative or adjunct to corticosteroid and cytotoxic drugs in the management of patients with acute SLE. The benefit from plasmapheresis is based on the theory that inflammation can be reduced by removal of immune complexes and/or autoantibodies. While uncontrolled clinical trials suggest the benefit of plasmapheresis in SLE, alternative explanations are: 1) the beneficial effects of hospitalization; 2) the highly variable course of each patient, regardless of therapy; 3) the placebo effect of a very dramatic procedure. To determine the true advantage of removal of plasma we propose a comparison of two groups of patients differing only in whether or not plasma is removed.

Methods Employed:

Twenty patients with SLE meeting predefined clinical criteria for entry into the study will be randomized to undergo either 4 liter plasmapheresis or reinfusion apheresis. The patient will receive their randomized therapy 3 times a week for 2 weeks. Each reinfusion plasmapheresis will process a blood volume equivalent to 4 liters of plasma. In contrast, the plasmapheresed patients will not be reinfused with their separated plasma; they will receive volume-for-volume replacement with 5% albumin in normal saline. A clinical examiner unaware of the type of assigned therapy will perform several assessments of lupus activity during apheresis and for 4 weeks following the procedures. Serum levels of antibodies to DNA circulating immune complexes, and immunoglobulins will also be assessed for the 6 week period of therapy and follow-up.

Major Findings:

To date 20 patients have been randomized and complete data on 16 are available. Nine patients were randomized to receive plasmapheresis and seven patients were randomized to receive reinfusion apheresis. One patient from each wing was declared a treatment failure prior to the completion of 6 procedures. Plasmapheresis produced reductions in serum levels of antibodies to DNA, circulating immune complexes and immunoglobulins. The serologic changes tended to return to baseline 4 weeks following apheresis without "hyperrebound." No adverse clinical effects of plasmapheresis were detected. The changes in clinical desease activity were of similar degree in SLE patients receiving either plasmapheresis or reinfusion apheresis.

Significance to Biomedical Research and Program of the Institute:

The study has shown that a brief course of plasmapheresis has distinct effects on serologic measures of lupus activity but has no advantage over placebo in the amelioration of clinical features in patients with mild to moderate SLE. Further investigation regarding the efficacy of plasmapheresis in a more intensive setting (eg. longer courses of plasmapheresis or combination of plasmapheresis with other modalities) should be carried out in a controlled fashion.

Proposed Course:

A manuscript reporting the findings of the study is in preparation. We do not intend to extend these studies further at this time. The procedure will occasionally be used to secure large volumes of plasma samples from consenting patients.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41056- 01 ARB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Immunosuppressive Drug Therapy in Lupus Glomerulonephritis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Balow	Senior Investigator	ARB:NIADDK
Other:	J. L. Decker	" "	" "
	J. H. Klippel	" "	" "
	P. A. Plotz	" "	" "
	A. D. Steinberg	" "	" "
	R. L. Wilder	" "	" "

COOPERATING UNITS (if any)

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	-0-

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The efficacy of intensive, intermittent immunosuppressive drug therapy will be evaluated in patients with active lupus glomerulonephritis over a 30 month study period. Patients with renal biopsy documented active glomerulonephritis with or without renal functional deterioration will be treated with low dose corticosteroids and randomized to receive (a) intravenous methylprednisolone (1.0 gm/m^2 monthly for 6 months or (b) intravenous cyclophosphamide 1.0 gm/m^2 monthly for 6 months or (c) intravenous cyclophosphamide 1.0 gm/m^2 monthly for 6 months and then every 3 months for the remaining 24 months of the study. During the final 24 months of the study, all patients will receive low dose prednisone. Active disease, as manifested by renal functional deterioration, increased proteinuria or worsened urinary sediment, will be treated by increased doses of prednisone. Comparison will be made of the number of favorable outcomes of renal function and glomerular pathology and drug related toxicities achieved by each treatment group at the end of the 6th and 30th study months.

Project Description:

Objectives:

Although corticosteroids are the historical mainstay of treatment for lupus nephritis, their unfavorable side-effects have prompted continued search for modifications of doses and routines of administration to achieve higher therapeutic indices. Following widespread use in treatment of transplant rejection, intermittent high-dose pulse corticosteroids have been found to be effective in treatment of SLE and possibly less toxic than conventional high-dose daily corticosteroids. Search for improved therapeutic indices in treatment of lupus nephritis has also led to trials of immunosuppressive drugs. In clinical trials to date, there is no convincing evidence, however, that conventional oral cyclophosphamide or azathioprine add to the efficacy of corticosteroids alone. Preliminary results obtained in ongoing NIH Protocol #76A-140 suggest that intermittent pulse cyclophosphamide given intravenously every 3 months has a low incidence of side effects and possibly greater efficacy than conventional corticosteroids in treating lupus nephritis.

The present study seeks to evaluate regimens of corticosteroids and cyclophosphamide similarly administered by intravenous pulse doses to patients with active lupus nephritis, all of whom will be receiving a basic regimen of moderate-dose oral corticosteroids. In order to avoid the morbidity associated with conventional high-dose corticosteroids (eg. prednisone $\geq$ 1.0 mg/kg/day for 8-12 weeks), the regimen of pulse methylprednisolone described herein will be considered high-dose corticosteroid treatment. The patients receiving pulse corticosteroids in addition to baseline oral corticosteroids will be defined as the comparison group to which the experimental groups receiving pulse cyclophosphamide will be compared. The 2 different cyclophosphamide regimens were chosen to a) compare the relative efficacy of methylprednisolone and cyclophosphamide and b) to evaluate the efficacy of long term, essentially prophylactic maintenance cyclophosphamide therapy.

Methods Employed:

This study will be a prospective controlled study in which patients will be randomly allocated to one of 3 treatment groups (pulse methylprednisolone monthly for 6 months or pulse cyclophosphamide monthly for 6 months or pulse cyclophosphamide monthly for 6 months followed by pulse cyclophosphamide every 3 months for an additional 2 years).

The study will be analyzed at 6 and 30 months for the following outcome variables: 1) renal failure, 2) renal functional deterioration, 3) deterioration of renal biopsy pathology and 4) rates of major complications of treatment in each group.

Significance to Bio-Medical Research and the Program of the Institute:

Although the prognosis of lupus glomerulonephritis has impoved substantially over the past 2 decades, a continued search for more effacious and simultaneously less toxic drug therapy is needed in the management of this disease. Preliminary experience has been most encouraging in the use of high-dose intermittent drug therapy, as described in this protocol, in regard to maximizing efficacy and minimizing side effects.

Proposed Course:

This study is expected to enter 60 patients over a 4 year period each of whom will be treated and followed over a 30 month protocol period.

Publications:

None

METABOLIC DISEASES BRANCH

The general goals of the Branch are to investigate the structure, secretion and biochemical mechanism of action of hormones governing ion transport and mineral metabolism and to apply the knowledge gained to understanding and treating related clinical disorders. Current investigations include hormone-receptor interaction, mechanism of receptor and guanine nucleotide control of adenylate cyclase, cyclic AMP control of ion transport, and regulation of parathyroid hormone secretion. Systems under study include β-adrenergic receptors in several tissue, isolated parathyroid cell systems and skeletal and renal tissue. Clinical studies on hyperparathyroidism and pseudohypoparathyroidism continue to utilize extensively the research findings from the laboratory. Radioimmunoassays for parathyroid hormone have been applied to determine the hormone in samples obtained selectively by venous catheterization, thereby allowing localization of parathyroid adenomas. Further studies make use of urinary cyclic AMP clearance as a clinical parameter of parathyroid hormone action. Another investigation takes advantage of the autologous parathyroid transplant program developed in collaboration with the Surgery Branch, NCI. Autologous transplants to the arm allow direct sampling of draining veins for parathyroid hormone secretion in man under conditions wherein the tissue can be selectively challenged with potential secretagogues or inhibitors of parathyroid secretion.

β-Adrenergic Receptor of the Avian Erythrocytes.

Interactions of catecholamines with β-adrenergic receptors and activation of adenylate cyclase are under study with the plasma membranes of turkey erythrocytes and other cell systems. Specific receptors have now been identified on turkey erythrocytes, parathyroid cells, pineal cells, rat, guinea pig and monkey lung membrane preparations and rat liver membranes.

β-Adrenergic receptor sites are identified using a radioiodinated β-blocker iodo-hydroxybenzylpindolol. A series of β-adrenergic agonists and inhibitors compete for binding with affinities paralleling biological effectiveness as activators or inhibitors of catecholamine-stimulated adenylate cyclase. Receptor occupancy for agonists correlates with function (cyclic AMP production). The stereospecificity of interaction with the iodinated β-blocking agent and the correspondence between affinity and biological effectiveness indicate that binding of iodohydroxybenzylpindolol validly reflects interaction with the β-adrenergic receptor.

Receptors for Calcitonin and Parathyroid Hormone

Studies in collaboration with Dr. T. Murray (St. Michael's Hospital, Toronto) have led to development of radiolabeled intact bovine parathyroid hormone as a ligand. This material binds to receptors in canine renal plasma membranes with kinetic properties distinct from those of radiolabeled synthetic amino terminal bioactive fragments. Thus the new ligand should prove useful for evaluating PTH-receptor interactions of the native hormone.
[Drs. Marx, Aurbach and Rizzoli]

Guanine Nucleotide Regulatory Protein

Studies on the role of guanine nucleotides in the control of adenylate cyclase activity are continuing. Guanine nucleotides enhance catecholamine-stimulated adenylate cyclase activity in turkey erythrocyte membranes. This action is mediated by a guanine nucleotide binding regulatory protein which is distinct from the receptor and the catalytic unit of adenylate cyclase. In the turkey erythrocyte membrane system, exchange of guanine nucleotides bound to the regulatory protein is strictly dependent on the action of hormone (β-adrenergic agonists). However, treatment of membranes with isoproterenol and GMP (a nucleotide bound with low affinity) permits later hormone-dependent activation and manipulation of the regulatory protein. After isoproterenol and GMP treatment, the guanine nucleotide regulatory protein is extracted from the membrane by Lubrol PX, and adsorbs to a GTP affinity matrix, from which it is eluted by high concentrations of guanine nucleotides. The regulatory protein, purified 50-100 fold by affinity adsorption, restores both guanine nucleotide- and fluoride-responsive adenylate cyclase activity to membranes from a mutant (CYC^-) strain of S49 mouse lymphoma which lacks the regulatory protein.

The guanine nucleotide regulatory protein from turkey erythrocytes is covalently radiolabeled by the cholera toxin-catalyzed transfer of $[^{32}P]$ADP-ribose from $[^{32}P]$NAD to a 42,000 M_r subunit of the regulatory protein. $[^{32}P]$ADP-ribosylation permits detection of the regulatory protein during detergent extraction, affinity purification, sucrose gradient centrifugation, and manipulations which cause inactivation of the regulatory protein. Radiolabeling of the regulatory protein should permit more accurate quantitation of enrichment during purification. Similar techniques applied to human tissue may permit detection of quantitative and qualitative alteration in hormone-resistant states such as pseudohypoparathyroidism.

Changes in the nature of the guanine nucleotide bound to the regulatory unit influence fluoride-stimulated adenylate cyclase activity. Incubation of turkey erythrocyte membranes with isoproterenol and GMP clears many of the regulatory sites of tightly bound GDP, substituting GMP. Fluoride-stimulated adenylate cyclase activity declines during isoproterenol and GMP treatment, but is restored by addition of GTP or GDP. Studies performed with the GDP analogs $GP(CH)_2P$ and GDP- -S, and direct analysis of the nucleotides bound at isoproterenol-dependent regulatory sites during fluoride activation, indicate that fluoride does not enhance conversion of GDP to GTP at regulatory sites, nor does it inhibit hydrolysis of GTP to GDP. Also, exchange of guanine nucleotide bound to the regulatory protein with nucleotides in the medium does not occur during enzyme activtion by fluoride.

Studies with partially purified, affinity eluted, guanine nucleotide regulatory protein confirm that fluoride stimulation of adenylate cyclase depends on the guanine nucleotide bound to the regulatory unit. Regulatory protein eluted with either GTP or GDP restores fluoride-stimulated enzyme activity to the S49 CYC^- acceptor, but regulatory protein eluted with GMP restores fluoride-stimulated activity only when GTP or GDP were added, indicating that the guanine nucleotide regulatory protein, and an appropriate nucleotide, are necessary for fluoride-stimulated adenylate cyclase activity.

Cholera toxin catalyzes [^{32}P]ADP-ribosylation of the 42K subunit of the G unit. Limited proteolysis generates fragments detected by autoradiography after SDS-gel electrophoresis. Unique proteolytic fragments are generating depending upon whether an active (e.g. GTP-γ-S) or inactive (e.g. GDP) guanine nucleotide is bound to the G unit. Identical results are obtained with membranes possessing the catalytic unit and with affinity eluate. lacking catalytic unit. These results point to a guanine nucleotide-directed change in conformation of the G unit and correspond to changes in apparent size of the G unit (as assessed by sucrose density gradient centrifugation) induced by GDP vs GTP-γ-S.
[Drs. Spiegel, Downs, Levine and Aurbach, NIADDKD]

Vanadate, an anion with important effects on Na, K-ATPase, also stimulates adenylate cyclase. We have shown that as with fluoride, stimulation by vanadate requires the G unit. Vanadate and fluoride stimulation differ, however, in their susceptibility to inhibition by cholera toxin and the GDP analog GDP-β-S.

The mechanism of calcium inhibition of adenylate cyclase was studied using partially separated components of the enzyme complex and analysis of free calcium concentration. The K_i for inhibition of the enzyme by free calcium was 2.48×10^{-4} M. Calcium did not affect hormone-stimulated guanine nucleotide exchange, conformation of the activated regulatory (G unit) protein or of the catalytic unit. Neither did calcium affect dissociation of activated G unit from catalytic unit nor convert activated catalytic unit to an inactive form. Evidence was found that calcium inhibits the catalytic subunit of adenylate cyclase by direct competition with manganese for its regulatory site.

Pseudohypoparathyroidism

The molecular defect in pseudohypoparathyroidism (PHP) has been investigated using techniques adapted from basic studies on the turkey erythrocyte adenylate cyclase complex. Patients with PHP can be divided into two phenotypes, i.e. those showing features of Albright's hereditary osteodystrophy (+AHO) and those lacking such features (-AHO). We have assayed the guanine nucleotide regulatory unit (G unit) in red cell membranes from about 30 patients with PHP and found that +AHO patients generally show a 50% reduction in functional G unit while -AHO patients show a normal G unit complement. This finding suggests that in PHP +AHO patients a deficiency in functional G units affects all cells and should cause generalized hormone resistance rather than resistance to PTH alone. Evidence confirming this hypothesis has been obtained in *in vivo* studies of patients with PHP + and -AHO. The former, for example, show resistance to TSH and glucagon while the latter in general do not.
[Drs. Spiegel, Levine, Downs, Marx and Aurbach]

Primary Hyperparathyroidism and Familial Hypercalcemia

Clinical studies are continuing on primary hyperparathyroidism and its familial variants. Detailed family screening and case finding has produced 41 kindreds for analysis. These studies have allowed segregation of the familial variants into two apparently distinct disease syndromes - familial multiple endocrine adenomatosis (MEN I) and familial hypocalciuric hypercalcemia (FHH). FHH was distinguished from MEN I by 1) virtually a 100% penetrance for hypercalcemia before age 20, 2) milder clinical manifestations - low incidence of recurrent nephrolithiasis or recurrent peptic ulceration, 3) no hypercalciuria, 4) normal basal concentrations of gastrin, and 5) poor response to subtotal parathyroidectomy. Distinction between the two syndromes, both inherited as autosomal dominant traits, is important because in FHH the clinical course is generally milder and subtotal parathyroidectomy is less likely to be beneficial. FHH accounts for approximately 10% of all unsuccessful parathyroidectomies in hypercalcemia. In FHH the ionized and ultrafiltrable calcium concentration in serum is elevated in proportion to the increase in total calcium. In these patients the filtrable load of calcium is high in association with a marked decrease in renal calcium clearance. The concentration of parathyroid hormone in plasma is lower in patients with FHH than in typical primary hyperparathyroid patients with similar degrees of hypercalcemia whether assessed by PTH radioimmunoassay or by renal clearance of cAMP or phosphate. The parathyroid glands show hyperplasia in most cases. The plasma and urine cAMP response to PTH infusion is greater in FHH than in typical primary hyperparathyroidism. Even when these patients become surgically hypoparathyroid, the low renal clearance of calcium is strikingly persistent during calcium infusion. [Drs. Marx, Spiegel, Downs, Levine, Lasker and Aurbach]

Studies on modes of localizing parathyroid tumors continue. A high degree of success has been obtained in localizing tumors through vascular catheterization procedures. Parathyroid arteriography developed and performed by Dr. John Doppman afforded, in approximately 45% of cases tested, the identification of abnormal masses of tissue subsequently identified as parathyroid. Confirmation of the mass thus identified as parathyroid tissue can be accomplished by verifying high concentrations of parathyroid hormone by radioimmunoassay in veins draining the lesion. Draining vessels contain concentrations of hormone 2- to 20-fold or more greater than the concentration of parathyroid hormone detected by immunoassay in the peripheral plasma. In cases with single adenomas, this concentration ratio is greatest selectively in the one vein draining the lesion. This technique utilizing specific radioimmunoassays and selective catheterization is applicable also to determining the source of hormone in ectopic hyperparathyroidism produced by non-parathyroid cancers. Localization of anterior mediastinal parathyroid adenomas has become possible using computed tomography. Eight mediastinal adenomas have been treated nonsurgically by percutaneous injection via catheter occlusive agents into the arterial blood supply with 7 complete and one partial remissions.

A new series of radioligands for parathyroid hormone radioimmunoassay has been developed, using synthetic sequences and analogs from the midregion of human PTH. These reagents have led to development of sensitive and practical radioimmunoassays. Using these assays and gel filtration of plasmas, we have demonstrated that certain hyperfunctioning parathyroid glands release a previously unrecognized large PTH fragment containing the midregion but deficient in carboxyterminal immunoreactivity.

Although results with parathyroid radionuclide imaging after intravenously administered [^{75}Se]-selenomethionine have been disappointing, we have been

obtaining parathyroid scans after administering this isotope intraarterially during parathyroid arteriography. Several cases wherein cannulation of most of the important vessels potentially supplying the parathyroids was possible have shown correctly localizing scans although careful interpretations are necessary to avoid false positives. This technique appears to be especially useful in those patients with juxta- or intra-thyroidal adenomas even when arteriography is ineffective. Radionuclide images are often positive because suppression of the thyroid with triiodothyronine prior to scanning improves the specificity of the parathyroid scan but not of the arteriogram.
[Drs. Marx, Spiegel, Downs, Stock and Aurbach, NIADDKD: Dr. Doppman, Radiology Department, C.C.: Dr. L.E.Mallette, V.A.Hospital, Houston, Texas]

We have studied a patient with parathyroid carcinoma who showed markedly elevated serum concentrations of the glycoprotein hormone subunits hCG-α and hCG-β, which fell dramatically after surgical removal of malignant tissue. Screening of a large number of patients with different forms of hyperparathyroidism has shown these subunits to be a marker for malignancy in parathyroid disease or for islet cell involvement in familial multiple endocrine adenomatosis (MEN I).
[Drs. Marx, Spiegel, Downs and Aurbach, MDB, NIADDKD; Drs. Weintraub and Rosen, CEB, NIADDKD]

The role of $1,25(OH)_2D_3$, the most potent natural metabolite of cholecalciferol, has been assessed in hypocalcemic states. We have discovered a family with a unique disturbance of calciferol metabolism. Two siblings have rickets and osteomalacia attributable to decreased target tissue sensitivity to $1,25(OH)_2D$. When maintenance of serum concentrations of $1,25(OH)_2D$ with appropriate calciferol analogs at concentrations approximately 20-fold normal, allows full control of the disorder. One of these patients was monitored during the course of her first pregnancy. The fetus developed normally even though $1,25(OH)_2D_3$ levels were extraordinarily high in maternal and cord blood.
[Drs. Marx, Spiegel and Downs, NIADDKD: Dr. C. Eil, U.S.Naval Medical Center: Drs. Hamstra and DeLuca, University of Wisconsin]

Cultured fibroblasts from normal human skin take up $1,25(OH)_2D_3$ into the nucleus via a process mediated by specific receptors. Normal cells have a mean of 10,000 nuclear receptor sites per cell.

Analogous studies with cells cultured from four members of two families (one family manifesting the disease together with total alopecia) with target insensitivity to calciferol revealed grossly deficient nuclear uptake.
[Drs. Marx, Liberman, Eil, Hamstra, DeLuca and Rosen]

Skin fibroblasts were cultured from patients with 1,25-dihydroxy D[$1,25(OH)_2D$] resistant osteomalacia. The cultured cells were tested for uptake of labeled $1,25(OH)_2D$. Uptake was markedly reduced in cells from affected subjects. A $1,25(OH)_2D$ receptor defect seems to explain vitamin D metabolite-resistant osteomalacia in these cases.
[Drs. Marx, Liberman and Eil]

Rapid determination of intraoperative U_{cAMP} excretion (using the Gammaflo machine for <7 min cAMP radioimmunoassay) has proven to be a valuable tool in guiding surgery for primary hyperparathyroidism. Persistent elevation of U_{cAMP} requires continued search for abnormal tissue even after 1 or more abnormal glands have been removed. A rapid (mean 1.5 hours) drop in U_{cAMP} to the normal range obviates the need for continued exploration even in cases where histologic confirmation of parathyroidectomy is lacking. Spurts in U_{cAMP} above baseline may provide a clue to the location of abnormal parathyroid tissue.
[Drs. Spiegel, Marx, Downs and Aurbach, NIADDKD: Drs. Saxe and Brennan, NCI Surgery]

Determination of urinary cAMP excretion postoperatively in patients undergoing neck exploration for primary hyperparathyroidism is a useful method for assessing postoperative parathyroid function. U_{cAMP} excretion declines postoperatively in all patients in whom hypercalcemia is corrected but not in those with persistent hypercalcemia. In patients becoming severely hypocalcemic (and requiring vitamin D therapy) postoperatively, U_{cAMP} measurement enables one to distinguish patients with decreased parathyroid reserve as the cause for hypocalcemia (low U_{cAMP} excretion) from patients with healing osteitis fibrosa ("hungry bones") as the basis for hypercalcemia. U_{cAMP} in the latter group is often elevated but can be suppressed if serum calcium is normalized. Elevated U_{cAMP} excretion postoperatively in the face of hypocalcemia enables one to predict that vitamin D therapy will be required temporarily and precludes the need for parathyroid autografts.
[Drs. Spiegel, Marx, Downs, Levine, Lasker and Aurbach, NIADDKD]

Studies have continued with dispersed bovine and human parathyroid cells. PTH secretion from parathyroid glands _in vivo_ and cells _in vitro_ is inhibited by calcium. Calcium may also modulate synthesis and degradation of PTH. This control exerted by calcium may be changed in certain pathologic states (glandular adenomas, carcinomas and perhaps hyperplasia). Several earlier studies have strongly suggested that cAMP is an intracellular mediator of PTH secretion; agents which alter cellular cAMP change PTH secretion in the same direction. Calcium decreases cellular cAMP although some of its effects may be independent of cAMP. The adenylate cyclase of the parathyroid cell is a potential regulatory site for the action of calcium. Adenylate cyclase in homogenates of dispersed parathyroid cells shows sensitivities to Ca (1/2 maximal inhibition at approximately 0.2 mM added calcium) that are similar in human parathyroid adenomas, carcinomas or hyperplastic glands and like that of normal bovine glands. There was no correlation between the _in vitro_ calcium suppressibility of PTH release by dispersed cells (several of the adenomas and the one carcinoma were not suppressed by high extracellular calcium) and the sensitivity of the adenylate cyclase in the broken cell preparation. This suggests that the adenylate cyclase responsiveness to calcium is not the site of derangement in these pathological states.

Dopamine-responsive adenylate cyclase was identified and studied in a particulate preparation of bovine parathyroid cells. Dopaminergic-specific agonists and antagonists and not adrenergic agents selectively interacted with this dopamine-responsive system. These studies established that dopamine-mediated cAMP generation and parathyroid hormone release are affected by stimulating adenylate cyclase in the bovine parathyroid cell.
[Drs. Attie, Brown, Gardner, Spiegel and Aurbach]

Humoral Hypercalcemia of Malignancy

A Leydig cell transplantable tumor causes hypercalcemia in rats. cAMP is increased in the urine of the tumor-bearing animals but plasma PTH is undetectable. Thus hypercalcemia apparently can be attributed to a non-parathyroid tumor factor that may, like parathyroid hormone, act through the adenylate cyclase-cyclic AMP system. This humoral factor, whose identity we wish to establish, presumably causes hypercalcemia by resorption of bone. Tumor cells cultured *in vitro* elaborate into the medium, a bone resorbing substance.
[Drs. Spiegel, Saxe, Deftos, Heersche, Downs, Marx and Aurbach]

Clinical Studies in Osteoporosis

The cooperative study in Jerusalem of 300 ambulatory patients with osteoporosis is nearly complete. Preliminary analysis of the trial of high calcium, high phosphate and sodium fluoride has revealed the following: a decrease in bone density was observed in 30 percent of the osteoporotic patients treated with placebo for 3 years; no decrease in bone density was observed in the osteoporotic patients receiving $CaHPO_4$ and NaF for 2 years, and in 19 percent of this group slight increase in bone density was seen.

The Physics Department of Hadassah Medical Organization has been involved in developing the relatively new technique of Compton Scattering for measurement of bone density and has made a comparative study of it with photon absorptiometry. For Compton Scattering, the coefficient of variation among individuals was less than for photon absorptiometry, and the Compton Scattering technique detected a significant difference in bone density between osteoporotic and non-osteoporotic patients.
[Drs. Aurbach, Whedon, Marx, Spiegel and Shapiro]

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43001-16 MD

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies in Bone Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD
G. D. Whedon, M.D., Director, NIADDKD
S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
J. Shapiro, M.D., Associate Director, Clinical Center, NIH.

COOPERATING UNITS (if any)
Osteoporosis and Fracture Research Institute, Hadassah Medical Organization, Jerusalem, Israel

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK , NIH, Bethesda, Md. 20205

TOTAL MANYEARS: .25	PROFESSIONAL: .25	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of the project on bone metabolism are to investigate the factors influencing mineral storage and loss in demineralizing bone diseases and to study the influence of added calcium and phosphate on resorption of bone mineral. The project involves estimation of bone density by photon absorptiometry and various techniques relative to vitamin D and fluoride metabolism.

During the past year, clinical studies of osteoporosis have been continued, using photon absorptiometry and Compton Scattering to determine the possible effect of dietary manipulation on bone density. Preliminary analysis of the data suggests a slight positive effect on bone density from di-calcium phosphate and sodium fluoride.

PHS-6040
(Rev. 2-81)

Objectives: To investigate the factors influencing mineral storage and loss in demineralizing bone diseases.

Methods Employed: (1) Estimation of bone density in the radius by photon absorptiometry and by Compton Scattering. (2) Estimation of intestinal absorption of calcium by oral administration of tracer doses of ^{47}Ca. (3) Tests of fluoride tolerance.

Major Findings: The cooperative study between investigators in NIADDK and members of the staff of Hadassah Medical School-Hebrew University involving approximately 300 ambulatory patients with osteoporosis in Jerusalem is now in its eighth year and nearing completion. Accession of new patients has been stopped. By a combination of dietary survey, X-ray, bone mineral content, medical history and other methods, considerable information on the natural history of osteoporosis is anticipated; in addition, a double-blind study is being made of the possible value of oral administration of high calcium, high phosphate and fluoride in treatment of osteoporosis. At the dosage of fluoride being used, 20 mg F per day, undesirable side effects have been very low. Although the number of patients either medically unfit for the study or unwilling to cooperate has been much higher than expected, once entered into active study the drop-out rate has been moderate. Preliminary analysis of the data has revealed the following: a decrease in bone density was observed in 30 percent of the osteoporotic patients treated with placebo for 3 years; no decrease in bone density was observed in the osteoporotic patients receiving $CaHPO_4$ and NaF for 2 years, and in 19 percent of this group slight increase in bone density was seen.

In association with this project, members of the Physics Department, Hadassah Medical Organiztion, are being supported in the development of a relatively new technique of bone densitometry, Compton Scattering, which it is hoped may be applied to the spine, a most difficult location in which to make such measurements, yet the most critical for assessment of the status of osteoporosis. A comparative study has been made in the distal radius of this technique (BD) with bone mineral content (BMC) measured by photon absorptiometry. The coefficient of variation fron individual to individual was low in BD, higher with BMC. In comparing measurements by the two methods between non-osteoporotic subjects and definitely osteoporotic subjects (diagnosed by clinical grading of radiographs), the difference between the two groups was highly significant by BD and not significant by BMC.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43002-16 MD

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Structure, Secretion and Mechanism of Action of Parathyroid Hormone

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. D. Aurbach, M.D., Chief, MDB, NIADDKD
S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
M. A. Levine, M.D., Clinical Associate, MDB, NIADDKD
R. Lasker, M.D., Clinical Associate, MDB, NIADDKD
M. F. Brennan, M.D., Surgery Branch, National Cancer Institute
J. T. Potts, Jr., M.D., Massachusetts General Hospital, Boston
H. T. Keutmann, M.D. "
M. Rosenblatt, M.D. "
T. Murray, M.D., Visiting Scientist, MDB, NIAMDD
R. Rizzoli, M.D., Visiting Scientist, MDB, NIAMDD

COOPERATING UNITS (if any)

Endocrine Unit, Massachusetts General Hospital

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK , NIH, Bethesda, Md. 20205

TOTAL MANYEARS: 4.00	PROFESSIONAL: 3.00	OTHER: 1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There is still much to be learned about the nature of circulating parathyroid hormone in clinical disease. It is the purpose of this project to study the function and mechanism of action of parathyroid hormone, study its relationship to human disease, and develop clinically useful tests for circulating parathyroid hormone. From these studies it is expected that one can understand the pathophysiology of certain metabolic diseases of bone and endocrine disturbances. The entire structures of bovine, porcine and human parathyroid hormone have been determined. Synthetic polypeptides representing the first 34 residues of bovine and human parathyroid hormone have been prepared. These molecules show all the biological properties of the native hormonal polypeptide. Highly sensitive radioimmunoassays for the hormone have been developed and are being modified further for improved clinical diagnostic parameters. Studies show that the mechanism of action of the hormone is mediated through direct hormonal activation of adenylate cyclase in bone and kidney. An isolated parathyroid cell system has been developed that allows studies of secretagogues for parathyroid hormone.

Objectives: To study chemical properties and structure of parathyroid hormone; to relate chemical properties and structure to biological activity and to study the mechanism of action of the hormone; to develop valid radioreceptor assays for the hormone; to develop clinically useful tests for circulating parathyroid hormone; to study the physiological regulation of secretion of the hormone.

Methods employed: The hormone is assayed in vivo in rats, in vitro by measuring hormonal activation of adenylate cyclase, or by a highly sensitive radioimmunoassay. Adenylate cyclase is measured by determining conversion of α-^{32}P-labeled ATP to cyclic 3',5'-AMP. The hormone is iodinated by electrolytic or enzymatic methods. Endogenous concentration of cyclic 3',5'-AMP or cyclic 3',5'GMP is determined by radioimmunoassay.

Major findings: mechanism of action. Our earlier studies showed that the parathyroid status of animals is an important factor regulating excretion of cyclic AMP in the urine. This finding led to development of the thesis that cyclic AMP is involved in the mediation of parathyroid hormone action. Subsequent work showed that parathyroid hormone in vitro activates adenylate cyclase in the kidney as well as in bone, the two organs known to be physiological receptors for action of the hormone. Adenylate cyclase in the kidney is found in two different anatomic zones; adenylate cyclase in the cortex responds specifically to parathyroid hormone whereas that in the medulla responds to vasopressin. This work and numerous corroborative studies in other laboratories established that the receptor for parathyroid hormone in kidney resides within the renal cortical tubule as had been suspected but not proven from indirect in vivo physiological experiments. Recent studies suggest that it is possible to iodinate parathyroid hormone electrolytically to provide a useful ligand for use in radioreceptor systems. Using this biologically active radioligand, receptors for native bPTH-(1-84) have been characterized in purified canine renal cortical membranes. These receptors are saturable, specific and coupled to the adenylate cyclase enzyme. Moreover, direct evidence with this system indicates that cleavage of parathyroid hormone aminoterminal to residue 43 is not required for binding in renal tissue.
[Drs. Murray, Rizzoli and Aurbach]

Human parathyroid hormone. Previous studies on this project led to complete analysis of the structure of human and bovine parathyroid hormones. Synthetic polypeptides, agonists as well as antagonists, are now available as a result of this research.

Studies have continued with dispersed bovine and human parathyroid cells. PTH secretion from parathyroid glands in vivo and cells in vitro is inhibited by calcium. Calcium may also modulate synthesis and degradation of PTH. This control exerted by calcium may be changed in certain pathologic states (glandular adenomas, carcinomas and perhaps hyperplasia). Several earlier studies have strongly suggested that cAMP is an intracellular mediator of PTH secretion; agents which alter cellular cAMP change PTH secretion in the same direction. Calcium decreases cellular cAMP although some of its effects may be independent of cAMP. The adenylate cyclase of the parathyroid cell is a potential regulatory site for the action of calcium. Adenylate cyclase in homogenates of dispersed parathyroid cells shows sensitivities to Ca (1/2 maximal inhibition at approximately 0.2 mM added calcium) that are similar in human parathyroid adenomas, carcinomas or hyperplastic glands and like that of normal bovine glands. There was no correlation between the in vitro calcium suppressibility of PTH release by dispersed cells (several of the adenomas and the one carcinoma were not suppressed by high extracellular calcium) and the sensitivity of the adenylate cyclase in the broken cell preparation. This suggests that the adenylate cyclase responsiveness to calcium is not the site of derangement in these pathological states.

Dopamine-responsive adenylate cyclase was identified and studied in a particulate preparation of bovine parathyroid cells. Dopaminergic-specific agonists and antagonists and not adrenergic agents selectively interacted with this dopamine-responsive system. These studies established that dopamine-mediated cAMP generation and parathyroid hormone release are effected by stimulating adenylate cyclase in the bovine parathyroid cell. [Drs. Attie, Brown, Gardner, Spiegel and Aurbach]

Significance to Biomedical Research and Program of the Institute: Parathyroid hormone controls the biology of skeletal tissue, mineral metabolism and renal function. These control mechanisms are directly related to programs of the Institute on renal disease, hyperparathyroidism, vitamin D metabolism, osteoporosis, and other metabolic bone diseases. This project since its inception has yielded significant new information and insights relative to these Institute programs. The discovery that cyclic AMP is the mediator of parathyroid hormone action led to the elucidation of cyclic AMP as an important controlling biochemical intermediate in bone resorption mediated by a variety of biologically important factors. This discovery similarly led to important new clinical laboratory tests for hyperparathyroidism, pseudohypoparathyroidism and other disorders of parathyroid secretion and function. There are also recent suggestions from other institutions that parathyroid hormone may be involved in the pathogenesis and pharmacological management of osteoporosis.

Proposed Course of Project: Studies with isolated parathyroid cells will continue in a search for factors that control parathyroid hormone secretion. The mechanism whereby calcium controls cell function will also be examined with the isolated parathyroid cell preparation. Studies will continue in attempts to delineate the nature of circulating parathyroid hormone. Methods for identifying parathyroid hormone receptors in target tissues will continue to be explored. Intense interest persists in the application of knowledge from these projects to problems of clinical disease.

Publications:

1. Brown, E. M., and Aurbach, G. D.: Role of Cyclic Nucleotides in Secretory Mechanisms and Actions of Parathyroid Hormone and Calcitonin. In Munson, P.L., Diczfalusy, E., Glover, J., and Olson, R. E. (Eds.): Vitamins and Hormones, Vol. 38. New York, Academic Press, 1980, pp. 205-256.

2. Aurbach, G. D., and Martin, T. J.: Introduction. I. Biosynthesis, Secretion and Actions of Parathyroid Hormone. In Cohn, D. V., Talmage, R. V., and Matthews, J. L. (Eds.): Hormonal Control of Calcium Metabolism. The Netherlands, Excerpta Medica, 1981, pp. 3-4.

3. Aurbach, G. D.: Regulation of Secretion of Parathyroid Hormone. In Hamburger, J., Crosnier, J., and Funch-Bretano, J.-L. (Eds.): Actualites Nephrologiques de L'Hopital Necker. Paris, Flammarion Medecine-Sciences, 1981, pp. 7-20.

4. Brown, , E. M., Attie, M. F., Reen, S., Gardner, D. G., Kebabian, J., and Aurbach, G. D.: Characterization of dopaminergic receptors in dispersed bovine parathyroid cells. J. Mol. Pharmacol. 18: 335-340, 1980.

5. Attie, M. F., Brown, E. M., Gardner, D. G., Spiegel, A. M., and Aurbach, G. D.: Characterization of the dopamine-responsive adenylate cyclase of bovine parathyroid cells and its relationship to parathyroid hormone secretion. Endocrinology 107: 1776-1781, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43003-16 MD

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies on the Mode of Action of Thyrocalcitonin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD
S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK , NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.25	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose is to study the interaction of calcitonin with its specific receptor target organs. The current investigations should provide further insight into the structure function relationship in calcitonin. Calcitonin is a small polypeptide hormone and therefore lends itself well to studies using synthetic peptide fragments. The system is also useful for characterizing hormone receptors in kidney, bone and other tissues. Studies are in progress to characterize further the interaction of calcitonin with tissue receptors. It also will be of interest to solubilize the receptors and characterize them chemically.

PHS-6040
(Rev. 10-76)

Objectives: To study the interaction of calcitonin with its specific receptor target organs.

Methods Employed: The interaction of calcitonin with its receptors has been evaluated by determining adenylate cyclase as well as binding of radioiodinated calcitonin to membranes isolated from renal tissue and to lymphoid cells isolated from human and other species or grown in tissue culture.

Major Findings: A purification procedure for kidney membranes has been developed. It has been shown that the calcitonin receptor from the kidney is concentrated at the corticomedullary junction. The membranes which bind calcitonin and contain calcitonin-sensitive adenylate cyclase show low specific activities for renal brush border enzymes, indicating that calcitonin receptors are localized at an anti-luminal aspect of renal cells or at a site that does not contain brush borders. Utilizing a series of calcitonin analogs, dose response curves were compared for binding to receptors from kidney and bone. The dose response curves in the two tissues are virtually superimposable, suggesting that the calcitonin receptor in the two organs has a similar molecular structure. The interaction of the hormone-receptor complex with the adenylate cyclase enzyme is modified by the presence of guanine nucleotides. In the presence of GMPPNP the threshold for hormonal activation is lowered.

The relative renal responses to calcitonin analogs are similar whether determined by competitive binding or adenylate cyclase activation. Thus potency differences of the analogs tested are largely dependent upon effectiveness at the receptor level. Activity of the adenylate cyclase enzyme of human lymphocytes was not modulated by ambient or bound calcitonin.

Calcitonin receptors were identified on cultured human lymphocytes. The characteristics of these receptors are similar to those on rat renal and calvarial membranes as determined by competitive binding studies. The lymphocyte receptors, however, do not appear capable of modulating adenylate cyclase enzyme in that cyclic AMP was not detected after adding calcitonin to intact cells. Lymphocyte preparations should prove useful, however, in that they represent a readily obtainable human tissue containing calcitonin receptors. Results with this human material indicated that, although calcitonin polypeptides have undergone remarkable evolutionary changes in structure and effectiveness, there has been no significant corresponding change in biological nature of calcitonin receptors.

Significance to Biomedical Research and Program of the Institute: The current investigations should provide further insight into the structure-function relationship in calcitonin. Calcitonin is a small polypeptide hormone and therefore lends itself well to studies using synthetic peptide fragments. The system is also useful characterizing hormone receptors present in kidney, bone, and other tissues.

Proposed Course of Project: Further studies are in progress to characterize further the interaction of calcitonin with its tissue receptors. It also will be of interest to solubilize calcitonin receptors and further characterize them. Purified lymphoid cell types are being characterized with respect to the density of calcitonin receptors on their surface. The whole cell system also may be applied to evaluate the regulation of receptor metabolism *in vitro* and in human disease states.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43004-16 MD

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies on pseudohypoparathyroidism and related disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD
S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
M. A. Levine, M.D., Clinical Associate, MDB, NIADDKD

COOPERATING UNITS (if any)

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK , NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.00	1.50	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In 1942 Albright and his associates described the features of a new clinical syndrome "pseudohypoparathyroidism" (PHP). Patients with this disorder differ from those with idiopathic hypoparathyroidism: they show characteristic constitutional features (Albright's hereditary osteodystrophy - AHO) and do not respond to exogenous parathyroid hormone. Subsequent to the original report, patients lacking the typical somatic features of AHO but resistant to endogenous and administered PTH have been described. We found previously that in PHP, U_{cAMP} (urinary cyclic AMP) does not increase normally in response to PTH administration. This indicated that there is a defective hormone receptor-adenylate cyclase complex in this disorder. We have now shown that red cells from patients with PHP + AHO are functionally deficient in a recently isolated protein ("G unit") that serves to couple hormone receptor to the catalytic unit of adenylate cyclase. Our results suggest that reduction in G units is the lesion responsible for hormone resistance in PHP + AHO and imply that the defect is not limited to PTH target organs (kidney and bone). Our recent clinical studies have corroborated this speculation, and redefine PHP with AHO as a disorder of generalized hormone resistance.

Project Description: In 1942 Albright and his associates described the features of a new clinical syndrome "pseudohypoparathyroidism." Patients with this disorder differ from those with idiopathic hypoparathyroidism in that they have characteristic constitutional features and they do not respond to exogenous parathyroid extract. Albright proposed that this disorder was attributable to lack of end organ sensitivity to normally-secreted endogenous parathyroid hormone. Our previous finding that patients with PHP lack the expected rise in U_{cAMP} excretion following PTH administration implicated a defective hormone receptor-adenylate cyclase complex as the basis for the disorder. Current studies have taken advantage of recent advances in understanding of the components of the receptor-cyclase complex and have attempted to pinpoint the component of the complex responsible for the clinically observed hormone resistance.

Methods:

1. Erythrocyte studies

Red cells were obtained by venipuncture and plasma membranes prepared by hypotonic lysis. The guanine nucleotide regulatory protein (G unit) was extracted with non-ionic detergents. Functional G units were assayed by combining human RBC extracts (source of G units) with catalytic units (either turkey RBC extracts or plasma membranes of CYC^- clone of S49 mouse lymphoma cells) and measuring resultant adenylate cyclase activity.

2. Skin fibroblast studies

Specimens of skin were obtained by 4 mm punch biopsy, and were grown to confluence in improved Eagles MEM with 10% FCS. Following 4-10 passages, fibroblasts were placed in microtiter wells and incubated to confluence. Intact fibroblasts were washed and incubated with serum-free MEM containing BSA, methylisobutyl xanthine and agonist. After hormone stimulation fibroblasts were lysed and total cAMP produced was measured by radioimmunoassay.

3. Clinical studies

Patients were admitted to the Clinical Center where the following provocative tests were performed: TRH stimulation (TSH, T_4, T_3, prolactin); glucagon stimulation (glucose, plasma cAMP); GNRF stimulation (LH, FSH); PTH infusion (urine cAMP excretion). In addition, a complete history and physical examination, skeletal radiographs and other tests were performed as appropriate. Genetic linkage studies were carried out on appropriate families.

Major Findings: Red cell membranes from patients with PHP and AHO show reduced G unit activity (mean reduction = 50%), by comparison with normal volunteers, and hyperparathyroid and surgical hypoparathyroid controls. The reduction in G unit activity was highly significant ($p < .001$) in each of two test systems (turkey erythrocyte soluble extracts and AC^- plasma membranes). We found no alteration in affinity for guanine nucleotide of the G unit from erythrocytes of patients with PHP/AHO. Only one (male) patient of 16 patients with PHP + AHO studied showed normal red cell G unit activity.

Cultured fibroblasts from four members of a kindred with PHP/AHO were examined for endogenous adenylate cyclase activity. Intact cells were stimulated with PGE_1 and PGE_2 and were shown to accumulate significantly less cAMP when compared to cultured fibroblasts from four normal lines (mean reduction ∿ 50%).

Red cell membranes from 15 patients with PHP but lacking somatic features of AHO were also examined for functional G unit activity, and in comparison to membranes from (+)AHO patients showed no defect.

Clinical studies of both groups of PHP patients (+AHO, -AHO) supported the hypothesis of a widespread disorder of hormone resistance in those patients who showed decreased erythrocyte G units (+AHO). These patients, in addition to PTH resistance, were found to have mild to moderate primary hypothyroidism (TSH resistance), abnormal hepatic responsiveness to glucagon (impaired hepatic adenylate cyclase response to glucagon), and a high incidence of reproductive abnormalities (primary or secondary amenorrhea).

Patients with normal RBC membrane G units (-AHO) in general showed no evidence of hormone resistance other than to PTH.

The mode of inheritance of this disorder remains unclear, and the recent delineation of patients into at least two subgroups based on erythrocyte G unit data further supports the concept of genetic heterogeneity. The findings of ∿50% reduction in G unit activity from 14/15 (+)AHO patients and the female preponderance is most compatible with sex-linked dominant inheritance, although autosomal dominant transmission is also possible. A recent report of a family with autosomal recessive transmission suggests that the disorder and its inheritance are quite heterogeneous.

Our findings suggest that reduction in function G units is the molecular basis for hormone resistance in most PHP patients with AHO. Since the G unit, unlike receptors for a given hormone (e.g. PTH), is not tissue-specific, but rather universally found in all adenylate cyclase-containing cells, one would anticipate that patients with PHP should show resistance to other hormones (TSH, glucagon, GNRF) whose action is mediated by cAMP, and our clinical findings corroborate this.

Significance to Biomedical Research and Program of the Institute: Our findings represent an important link between basic studies on the structure and function of the hormone receptor-adenylate cyclase complex and clinical studies of various endocrine disorders. While PHP itself is a rare disease, it provides an important model for the study of normal and abnormal hormone action. Our studies suggest that PHP may represent the first example of a human disease involving a defect in the G-unit protein only recently isolated and characterized. As such, PHP provides an important opportunity to assess the functional significance of this protein, the G unit in humans. Better understanding of the function of the G unit should increase our ability to define the pathophysiology of endocrine hyper- and hypofunctional states and may provide novel possibilities for therapeutic intervention.

Proposed Course of the Project: The immediate goals of our project are 1) to verify the reduction of funtional G units in tissues other than RBC, e.g. cultured fibroblasts (already under study) platelets from patients with PHP; 2) to identify the nature of the defect in the G unit in patients with PHP + AHO, i.e. structure vs regulatory mutation; 3) to sutdy the responsiveness of a variety of organs in patients with PHP to trophic hormones whose action is mediated by cAMP; 4) to delineate the pattern of inheritance of the disorder; 5) to attempt to elucidate the nature of the defect in PHP patients who show normal G unit activity.

Publications

1. Levine, M.A., Downs, R.W.,Jr., Marx, S.J., Lasker, R.D., Aurbach, G.D., and Spiegel, A.M.: Clinical and Biochemical Features of Pseudohypoparathyroidism. In Cohn, R.V., and Matthews, J.L. (Eds.): Hormonal Control of Calcium Metabolism. Amsterdam, Excerpta Medica, 1981, pp. 95-101.

2. Spiegel, A.M., Levine, M.A., Downs, R.W.,Jr., Aurbach, G.D., Marx, S.J., and Lasker, R.D.: Deficiency of hormone receptor-adenylate cyclase coupling protein: the basis for hormone resistance in pseudohypoparathyroidism. In Wilson, J. (Ed.): Receptors in Human Disease. American Physiological Society, in press.

3. Marx, S.J., Swart, E.G., Hamstra, A.J., and DeLuca, H.F.: Normal intrauterine development for the fetus of a woman receiving extraordinarily high doses of 1,25-dihydroxycholecalciferol. J. Clin. Endocrinol. Metab. 51: 1138-1142, 1980.

4. Eil, C., Liberman, U.A., Rosen, J.F., and Marx, S.J.: A cellular defect in hereditary vitamin D-dependent rickets type II: defective nuclear uptake of 1,25-dihydroxyvitamin D in cultured skin fibroblasts. N. Eng. J. Med. 304: 1588-1591, 1981.

5. Eil, C., and Marx, S. J.: Nuclear uptake of [^{3}H]1,25-dihydroxycholecalciferol in dispersed fibroblasts cultured from normal human skin. Proc. Natl. Acad. Sci. USA 78: 2562-2566, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER A01 AM 43005-16 MD

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Hormone Receptors, Adenylate Cyclase and Cyclic AMP-Mediated Ion Transport

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD
A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
M. A. Levine, M.D., Clinical Associate, MDB, NIADDKD
R. D. Lasker, M.D., Clinical Associate, MDB, NIADDKD
J. D. Gardner, M.D., Chief, Digestive Diseases Branch, NIADDKD
R. Horlick, MDB, NIADDKD

COOPERATING UNITS (if any)
Section on Gastroenterology, Digestive Diseases Branch, NIADDKD

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK , NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.25	2.50	2.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have studied the components of the hormone receptor-adenylate cyclase complex. The turkey erythrocyte, a readily available, homogeneous cell type has been used as a model system. The turkey RBC plasma membrane possesses β-adrenergic receptors, identified using radioiodinated hydroxybenzylpindolol, a high-affinity β-blocker. β-receptors are coupled to adenylate cyclase catalytic units by a guanine nucleotide binding protein (G-unit) which we have isolated by affinity chromatography. We have studied the role of the G unit in mediating activation of adenylate cyclase by hormone, fluoride and cholera toxin. β-adrenergic agonist stimulation of cAMP accumulation in turkey erythrocytes leads to significant changes in sodium and potassium transport.

Objectives: To isolate and purify the components of the hormone receptor-adenylate cyclase complex from particular tissues, to determine the nature of the interaction of the various components, and to determine the nature of the link between generation of cyclic 3',5'-AMP and solute transport in particular cell systems.

Methods Employed: Polypeptide and catecholamine hormones are purified and iodinated by standard methods or methods developed in the laboratory specifically for that purpose. Rate of association of hormones is determined by incubating plasma membrane fractions or whole cell with labeled hormones. The kinetics of binding is determined by rate measurements as well as assessment of steady state conditions. Specific hormone binding is defined in terms of dissociation constant (K_m) and the physiological characteristics of the system.

Adenylate cyclase is assayed in intact plasma membranes or in detergent extracts by monitoring conversion of [α-^{32}P]ATP to [^{32}P]cAMP. The G unit is assayed by combining it with catalytic units from turkey erythrocyte or the CYC^{-} clone of S49 mouse lymphoma cell plasma membranes and measuring the resultant increase in adenylate cyclase activity. The G unit is isolated from detergent extracts of turkey erythrocyte membranes by affinity chromatography on a GTP (γ-phosphate linked) matrix. Cholera toxin and [^{32}P]NAD are used to radiolabel covalently the G unit in various plasma membranes.

Major Findings: The G unit has been partially purified from turkey erythrocyte membranes with GTP affinity chromatography. Our studies show that the G unit is reversibly associated with the catalytic unit of the solubilized holoenzyme. The partially purified G unit from turkey erythrocyte restores both guanine nucleotide and fluoride responsive adenylate cyclase activity to homologous (turkey erythrocyte) and heterologous (CYC^{-} S49 mouse lymphoma cell line genetically deficient in G units) catalytic units.

A method has been developed to radiolabel covalently the G unit in turkey erythrocyte membranes. Cholera toxin catalyzes the transfer of [^{32}P]NAD to a subunit of the regulatory protein which has an apparent molecular weight of 42,000 on SDS gel electrophoresis. Labeling of the regulatory protein is cholera toxin specific, and is distinct from nonspecific labeling of other proteins which occurs without toxin. [^{32}P]ADP-ribosylation permits detection of the G unit during detergent solubilization, affinity purification, sucrose gradient centrifugation, and other manipulations. The radiolabeled 42,000 M_r peptide and the G unit (as measured by reconstitution of adenylate cyclase activity) coelute from the GTP affinity column upon addition of specific guanine nucleotides. Cholera toxin-mediated ADP ribosylation of the G unit alters turkey erythrocyte adenylate cyclase activity so that GTP becomes almost as effective as its non-hydrolyzable analogs, e.g. GTP-γ-S. A 42,000 M_r protein is also radiolabeled with cholera toxin and [^{32}P]NAD in human erythrocyte membranes. This correlates with the presence of functional G units in human red cells as measured by G unit assay.

Pigeon erythrocyte G units have also been radiolabeled using methods similar to those developed for turkey erythrocyte G units. G unit sediments more rapidly (4.0 S) in its basal, GDP-liganded state than in its activated, GTP-γ-S-liganded state (2.7 S). The susceptibility of ^{32}P-labeled pigeon G units to proteases varies depending on the guanine nucleotide bound to the G unit, indicating that the conformation of the G unit varies with the nature of the guanine nucleotide bound.

Vanadate, an anion with important effects on Na, K-ATPase, also stimulates adenylate cyclase. We have shown that as with fluoride, stimulation by vanadate requires the G unit. Vanadate and fluoride stimulation differ, however, in their susceptibility to inhibition by cholera toxin and the GDP analog GDP-β-S.

The mechanism of calcium inhibition of adenylate cyclase was studied using partially separated components of the enzyme complex and analysis of free calcium concentration. The K_i for inhibition of the enzyme by free calcium was 2.48×10^{-4}M. Calcium did not affect hormone-stimulated guanine nucleotide exchange, conformation of the activated regulatory (G unit) protein or of the catalytic unit. Neither did calcium affect dissociation of activated G unit from catalytic unit nor convert activated catalytic unit to an inactive form. Evidence was found that calcium inhibits the catalytic subunit of adenylate cyclase by direct competition with manganese for its regulatory site.

Significance to Biomedical Research and Program of the Institute: Studies of the structure and function of the components of the hormone receptor-adenylate cyclase complex increase our understanding of the mechanisms of hormone action. Recognition of the critical role of the G unit as a "coupling factor" in transmitting hormonal messages across cell membranes makes it likely that various disease states may involve G unit malfunction. Studies in our laboratory on the hormone resistance syndrome, pseudohypoparathyroidism, bear out this possibility (see separate PHP project report). G unit alterations may also lead to increased adenylate cyclase activity. This is best exemplified by the effects of cholera toxin. Indeed the severe secretory diarrhea seen in clinical vibrio cholera infection results from G unit ADP-ribosylation and consequent increased cAMP production in the gut. Fu r ther studies on the structure and function of the G unit may open up new possibiliies for therapeutic intervention in established cases of cholera. Labeling of the G unit with cholera toxin and [^{32}P]NAD may permit detection of quantitative or qualitative alterations in the G unit in various human disease states.

Proposed Course of Project: Future goals of this project include further purification of the G unit, production of antibodies to the G unit to aid in detection of genetic abnormalities in G unit function, study of the mechanism of coupling between the G unit and both hormone receptors and catalytic unit, and study of the relationship between the G unit and effects of guanine nucleotides on agonists (e.g. α-adrenergic, opiates, muscarinic) that act via inhibition of adenylate cyclase.

Publications

1. Aurbach, G. D.: Receptor-adenylate cyclase components: abnormalities in clinical medicine. Adv. Cyclic Nucleotide Res. 12: 1-9, 1980.

2. Nielsen, T. B., Downs, R. W., Jr., and Spiegel, A. M.: Restoration of guanine nucleotide and fluoride stimulated activity to an adenylate cyclase deficient cell line with affinity purified guanine nucleotide regulatory protein. Biochem. J. 190: 439-443, 1980.

3. Spiegel, A.M., Downs, R,W.,Jr., Levine, M.A., Singer, J.J.,Jr., Krawietz, W., Marx, S.J., Woodard, C.J., Reen, S.A., and Aurbach, G.D.: The role of guanine nucleotides in regulation of adenylate cyclase activity. Recent Progr. Hormone Res. 37: in press.

4. Aurbach, G. D.: Polypeptide and Amine Hormone Regulation of Calcium Fluxes. In Bronner, F. (Ed.): Calcium and Phosphate Across Biomembranes. New York, Academic Press, Inc., 1981, in press.

5. Aurbach, G. D., and Brown, E. M.: Hormone receptors, cyclic nucleotides, and control of cell function. In Freinkel, N. (Ed.): Contemporary Metabolism II. New York, Plenum Publishing Corporation, 1981, in press.

6. Downs, R.W.,Jr., Reen, S.A., Levine, M.A., Aurbach, G.D., and Spiegel, A.M.: Cholera toxin dependent ADP-ribosylation of the adenylate cyclase regulatory protein in turkey erythrocyte membranes. Arch. Biochem. Biophys. 209: 284- , 1981.

7. Spiegel, A.M., and Downs, R.W.,Jr.: Guanine nucleotides - key regulators of hormone receptor-adenylate cyclase interactions. Endocrine Rev. 2: in press, 1981.

8. Auerbach, D.A., Klein, D.C., Woodard, C., and Aurbach, G.D.: Neonatal rat pinealocytes: typical and atypical characteristics of [^{125}I]iodohydroxybenzylpindolol binding and adenosine 3',5'-monophosphate accumulation. Endocrinology 108: 559-567, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER A01 AM 43006-06 MD

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Study of Hyperparathyroidism: Etiology, diagnosis and treatment.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT
G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD
S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
A. M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
M. A. Levine, M.D., Clinical Associate, MDB, NIADDKD
R. D. Lasker, M.D., Clinical Associate, MDB, NIADDKD
A. C. Santora, M.D., Clinical Associate, MDB, NIADDKD
J. L. Doppman, M.D., Radiology Dept., Clinical Center
M. Brennan, M.D., and A. Saxe, M.D., Surgery Branch, NCI
J. T. Potts, Jr., M.D., Endocrine Unit, Massachusetts General Hospital
J. D. Gardner, M.D., Digestive Diseases Branch, NIADDKD
H. DeLuca, M.D., Department of Biochemistry, Univ. of Wisconsin

COOPERATING UNITS (if any)
Radiology Department, CC; Surgery Branch, NCI; Department of Biochemistry, University of Wisconsin; Endocrine Unit, MGH; Digestive Diseases Branch, NIADDKD

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK , NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.50	2.00	1.50

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project goal is the evaluation and treatment of hyperparathyroidism. Patients with persistent postoperative hyperparathyroidism are referred for evaluation and treatment. Hereditary hyperparathyroidism in particular is under investigation in the hopes of delineating hereditary molecular abnormalities in glandular regulation, as exemplified in the multiple endocrine neoplasia syndromes. Evaluation ranges from epidemiologic studies of families to in-house studies of patients and to in vitro analyses of excised tissue. Techniques currently being employed and improved include radioimmunoassay of parathyroid hormone, selective arteriography and selective venous sampling for parathyroid hormone, parathyroid gland cryopreservation and autotransplantation, and transcatheter parathyroid gland infarction.

PHS-6040
(Rev. 2-81)

Objectives: To study the regulation of parathyroid gland function. To develop and evaluate diagnostic tests of parathyroid function. To develop new methods for localization of hyperfunctioning parathyroid tissue. To evaluate methods of treatment of hyperparathyroidism.

Methods employed: Patients with suspected hyperparathyroidism are referred for evaluation and treatment. Evaluation includes radioimmunoassay of circulating parathyroid hormone, and of plasma and urinary cyclic AMP. Where appropriate, preoperative evaluation includes selective arteriography and venography with selective sampling for PTH determination and computerized tomography of neck and mediastinum. In selected cases adenomatous parathyroid glands are ablated by transarterial infusion of various agents. Parathyroid exploration is done by the Surgery Branch, NCI. Urinary cyclic AMP (U_{cAMP}) excretion is monitored during surgery, utilizing the gammaflo machine for rapid (<7 min) radioimmunoassay of cyclic AMP. If postoperative hypoparathyroidism is considered highly likely, parathyroid tissue may be simultaneously autotransplanted to a forearm muscle. Excised parathyroid tissue either fresh or cryopreserved is evaluated in vitro after preparation of dispersed cells. If postoperative hypoparathyroidism is considered possible, tissue is cryopreserved for subsequent autotransplantation.

Major findings: Diagnosis and clinical findings: A large number of patients with primary hyperparathyroidism have been treated and their clinical and chemical findings have been described. Weakness was noted more commonly than had previously been appreciated and muscle biopsy showed atrophy of type I and especially type II fibers in a manner diagnostic of denervating disease. Studies of neuromuscular function in subjects with secondary hyperparathyroidism showed similar clinical and histologic findings. The most useful diagnostic tests for hyperparathyroidism have been hypercalcemia associated with nonsuppressed or increased parathyroid hormone concentrations in peripheral blood and increased urinary excretion of cyclic AMP. These findings revert rapidly to normal following successful parathyroidectomy. Intestinal absorption of calcium is increased preoperatively and undoubtedly has a central role in causing hypercalcemia.

A new series of radioligands for parathyroid hormone radioimmunoassay has been developed, using synthetic sequences and analogs from the midregion of human PTH. These fragments have led to development of sensitive and practical radioimmunoassays. Using these assays and gel filtration of plasmas, we have demonstrated that certain hyperfunctioning parathyroid glands release a previously unrecognized large PTH fragment containing the midregion but deficient in carboxyterminal immunoreactivity. [G.D.Aurbach, S.J.Marx, A.M.Spiegel, R.W.Downs,Jr., M.A.Levine, R.Lasker, MDB, NIADDKD: A. Saxe and M. Brennan, Surgery Branch, NCI; L.E.Mallette, V.A.Hospital, Houston]

Although results with parathyroid radionuclide imaging after intravenously administered [^{75}Se]-selenomethionine have been disappointing, we have been obtaining parathyroid scans after administering this isotope intra-arterially during parathyroid arteriography. Several cases wherein cannulation of most of the important vessels potentially supplying the parathyroids was possible have shown correctly localizing scans although careful interpretations are necessary to avoid false positives. This technique appears

to be especially useful in those patients with juxta- or intra-thyroidal adenomas even when arteriography is inaffective. Radionuclide images are often positive because suppression of the thyroid with triiodothyronine prior to scanning improves the specificity of the parathyroid scan but not of the arteriogram.
[Drs. Marx, Spiegel, Downs, Stock and Aurbach, NIADDKD: Dr. Doppman, Radiology Department, C.C.]

We have studied a patient with parathyroid carcinoma who showed markedly elevated serum concentrations of the glycoprotein hormone subunits hCG-α and hCG-β, which fell dramatically after surgical removal of malignant tissue. Screening of a large number of patients with different forms of hyperparathyroidism has shown these subunits to be a marker for malignancy in parathyroid disease or for islet cell involvement in familial multiple endocrine adenomatosis (MEN I).
[Drs. Marx, Spiegel, Downs, and Aurbach, MDB, NIADDKD: Drs. Weintraub and Rosen, CEB, NIADDKD]

Several studies have utilized urinary cyclic AMP excretion as a parameter of parathyroid hormone action:

Our previous studies showed that intraoperative U_{cAMP} excretion fell rapidly to the normal range (mean = 1.5 hrs) after successful parathyroidectomy but failed to fall after unsuccessful surgery in patients with primary hyperparathyroidism (1°HPT). Availability of the gamma-flo machine (capable of performing cAMP radioimmunoassay in <7 min/sample) enabled us to study prospectively the utility of rapid intraoperative U_{cAMP} determination in guiding surgery for 1°HPT. The data from over 20 patients studied with this technique to date confirm the specificity of intraoperative U_{cAMP} excretion as an index of parathyroid gland function. Rapid U_{cAMP} determination proved to be extremely useful in several patients. A fall in U_{cAMP} to the normal range obviated the need for further neck exploration (with attendant increased morbidity) even when histologic confirmation of removal of abnormal parathyroid glands was lacking. Persistent (>1.5 hrs) elevation of U_{cAMP} signaled the need to continue exploration even after one or more enlarged glands had been removed. A peak in U_{cAMP} excretion above baseline was a clue to the location of an enlarged gland not found during previou exploration. This technique appears to be a valuable tool in assisting the surgeon exploring the particularly difficult group of patients who have undergone prior unsuccessful neck surgery.

Familial hyperparathyroidism. Surveys of families of patients with primary parathyroid hyperplasia show that hereditary disease is very common (approximately 50% in our series of 76 index cases with primary parathyroid hyperplasia). In fifteen kindreds a novel form of familial hypercalcemia was found wherein hypercalcemia occurs with hypocalciuria (FHH). Unlike typical cases of familial hyperparathyroidism, the penetrance of hypercalcemia approached 100% in the first two decades with severe expression even in one neonate. Review of results of surgery at the N.I.H. and elsewhere has shown that most patients in this category treated by subtotal parathyroidectomy had persistent postoperative hypercalcemia. In these families renal clearances of filtered calcium and magnesium are both lower than in typical primary hyperparathyroidism and circulating parathyroid hormone levels are lower than in typical primary

hyperparathyroidism. Circulating parathyroid hormone concentrations, assessed by three independent assay techniques, are lower in these patients than in patients with typical primary hyperparathyroidism. Furthermore, the cAMP response to infused PTH is exaggerated in these patients. In a series of 67 patients referred after exploration for hypercalcemia, six (9%) were previously unrecognized probands for kindreds with FHH. Analysis of urine calcium expressed as ratio of calcium clearance:creatinine clearance facilitates recognition of this disorder. Recognition of FHH in the population is important in order to obviate unnecessary parathyroid surgery in these cases. Quantitative analysis of histologic sections of parathyroid glands from 18 patients showed hyperplasia in at least 13.
[S.J.Marx, A.M.Spiegel, R.W.Downs,Jr., M.A.Levine, R.Lasker, and G.D.Aurbach, MDB, NIADDKD: J. Gardner, DDB, NIADDKD]

Application of new radiologic techniques: A series of studies has been carried out utilizing selective venous catheterization and a radioimmunoassay for parathyroid hormone for localization of parathyroid adenomas. A high degree of success has been obtained in localizing these tumors preoperatively. The catheter is passed through the femoral vein and thence through the major veins to reach the neck where selective samples are taken from each inferior and superior thyroid vein. Radioimmunoassays show that veins draining parathyroid adenomas frequently contain concentrations of hormone 10- to 20-fold or more greater than the concentration in the peripheral plasma. In cases with single adenomas, this concentration ratio is greatest selectively in one vein draining the adenoma. The contralateral vein shows concentrations equal to samples from the general circulation. Patients with hyperplasia of all four glands show concentrations of hormone in the thyroid veins on each side greater than concentrations in peripheral plasma. Usually in these cases the concentration ratio is not as high as for single adenomas. This technique utilizing specific radioimmunoassays and selective catheterization should be applicable also to determining the source of hormone in ectopic hyperparathyroidism produced by nonparathyroid cancers.

Thyroid and internal mammary arteriography enables preoperative identification of hyperfunctioning parathyroid tissue in 30% or more of cases and is also helpful in defining venous drainage to facilitate selective catheterization. In eight cases of mediastinal parathyroid adenoma treatment was accomplished by transarterial infusion of hypertonic glucose, gelfoam or autologous clot to occlude the catheterized parathyroid artery. Seven appear fully cured and one partially. There was no significant morbidity to these patients who would otherwise have required sternotomy.
[G.D.Aurbach, S.J.Marx, A.M.Spiegel, R.W. Downs, MDB, NIADDKD: Dr. J.Doppman, Radiology Department, C.C.]

Autotransplantation and cryopreservation of parathyroid: Persistent postoperative hyperparathyroidism or hypoparathyroidism are potential problems in patients with primary parathyroid hyperplasia or previous unsuccessful parathyroid exploration. These problems may be lessened if parathyroid tissue can be cryopreserved. We have developed experience with cryopreservation and autotransplantation of parathyroid glands in normal dogs and also in a few patients who developed permanent hypoparathyroidism postoperatively. Avoidance of tissue trauma or rapid changes in temperature or pH was essential

for tissue preservation. Canine parathyroid tissue has been successfully autotransplanted after cryopreservation for as long as nine months and function has been documented by measuring release of immunoreactive PTH and by induction of hypocalcemia following graft excision. Function of autografted parathyroid tissue _in vivo_ correlates well with assessment _in vitro_. One patient who received an autograft from a parathyroid adenoma developed rapid recurrence of primary hyperparathyroidism. Subsequently his tissue showed highly abnormal function _in vitro_ and _in vivo_. Graft excision was required and he became hyoparathyroid.
[G.D.Aurbach, S.J.Marx, A.M.Spiegel, R.W.Downs,Jr., M.A.Levine, and R.Lasker, MDB,NIADDKD; A. Saxe and M. Brennan, Surgery Branch, NCI]

Significance to Biomedical Research and Program of the Institute: As an outgrowth of these studies, methods of localizing and treating hypersecreting tissues of several types are being improved. Hyperparathyroidism is a prototype of endocrine hyperfunction. Some patients have hyperparathyroidism in combination with hereditary hyperfunction of other endocrine glands including pituitary and pancreas. These hyperfunctions are usually benign but occasionally become cancerous. By studying families with hereditary disease we hope to gain insight into specific molecular lesions that cause loss of normal cellular regulation in these tissues. This collaborative program offers comprehensive treatment for a select group of patients who have proven difficult or impossible to treat in other centers in the United States and abroad.

Proposed Course of Project: Patients with suspected primary hyperparathyroidism will be accepted for evaluation and treatment. We will continue to study familial patterns of hyperparathyroidism and efforts will continue towards improving techniques for assessment of parathyroid hormone by radioimmunoassay or bioassay. We will continue to explore new methods of localizing hyperfunctioning parathyroid tissue. The applicability of parathyroid embolization and the role of parathyroid autotransplantation will be investigated further. Patients in the entire program will provide a source of abnormal parathyroid glands to be studied in detail in vitro. Patients with parathyroid transplants will be ideal test subjects for putative parathyroid secretagogues.

Publications

1. Aurbach, G. D., Marx, S. J., and Spiegel, A. M.: Parathyroid hormone, calcitonin, and the calciferols. In Williams, R. R. (Ed.): _Textbook of Endocrinology_, Sixth Edition. Philadelphia, W. B. Saunders Company, in press.

2. Thorgeirsson, U., Costa, J., and Marx, S.J.: The parathyroid glands in familial hypocalciuric hypercalcemia. Human Pathology 12: 229-237, 1981.

3. Marx, S.J., Attie, M.F., Stock, J.L., Spiegel, A.M., and Levine, M.A.: Maximal urine-concentrating ability: familial hypocalciuric hypercalcemia versus typical primary hyperparathyroidism. J. Clin. Endocrinol. Metab. 52: 736-740, 1981.

4. Marx, S.J.: Familial hypocalciuric hypercalcemia. Editorial. N. Eng. J. Med. 303: 810-811, 1980.

5. Marx, S.J., Sharp, M.E., Krudy, A., Rosenblatt, M., and Mallette, L.E.: Radioimmunoassay for the middle region of human parathyroid hormone: studies with a radioiodinated synthetic peptide. J. Clin. Endocrinol. Metab. 53: 76-84, 1981.

6. Stock, J.L., Krudy, A.G., Doppman, J.L., Jones, A.E., Brennan, M.F., Attie, M.F., Downs, R.W.,Jr., Levine, M.A., Marx, S.J., Spiegel, A.M., and Aurbach, G.D.: Parathyroid imaging after intra-arterial injections of [^{75}Se]-selenomethionine. J. Clin. Endocrinol. Metab. 52: 835-839, 1981.

7. Spiegel, A.M., Eastman, S.T., Attie, M.F., Downs, R.W.,Jr., Levine, M.A., Marx, S.J., Stock, J.L., Saxe, A., Brennan, M.F., and Aurbach, G.D.: Intraoperative measurements of urinary cyclic AMP to guide surgery for primary hyperparathyroidism. N. Eng. J. Med. 303: 1475-1460, 1980.

8. Spiegel, A.M., Marx, S.J., Brennan, M.F., Brown, E.M., Downs, R.W.,Jr., Gardner, D.G., Attie, M.F., and Aurbach, G.D.: Parathyroid function after parathyroidectomy: evaluation by measurement of urinary cAMP. Clinical Endocrinology 15: in press.

9. Krudy, A.G., Doppman, J.L., Brennan, M.F., Marx, S.J., Spiegel, A.M., Stock, J.L., and Aurbach, G.D.: The detection of mediastinal parathyroid adenoma by CT, selective arteriography, and venous sampling: an analysis of 17 cases. Radiology, in press.

10. Krudy, A.G., Doppman, J.L., Marx, S.J., Spiegel, A.M., Aurbach, G.D., and Brennan, M.F.: Failure of ablation of a mediastinal parathyroid adenoma by repeated contrast staining. Radiology, in press.

11. Marx, S.J., Attie, M.F., Spiegel, A.M., Downs, R.W.,Jr., and Levine, M.A.: Familial hypocalciuric hypercalcemia: a disorder of calcium regulation in parathyroid glands and kidneys. In Cohn, D.V., Talmage, R.V., and Matthews, J.L. (Eds.): *Hormonal Control of Calcium Metabolism*. Amsterdam, Excerpta Medica, 1981, pp. 87-94.

12. Marx, S.J., Spiegel, A.M., Brown, E.M., Downs, R.W.,Jr., Attie, M.F., Stock, J.L., Levine, M.A., and Aurbach, G.D.: Urinary cyclic 3',5'-adenosine monophosphate (cAMP): an index of the action of parathyroid hormone on the kidney. Prog. Biochem. Pharmacol. 17: 182-189, 1980.

13. Marx, S.J.: Familial hypocalciuric hypercalcemia. Clin. Adv. Mineral Metab., in press.

14. Doppman, J.L., Brennan, M.F., Kahn, C.R., and Marx, S.J.: The circumscribing or peri-adenomal vessel: a helpful angiographic finding in certain islet cell and parathyroid adenomas. Am. J. Roent. 136: 163-165, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43007-01 MD

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Study of humoral hypercalcemia of malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Allen M. Spiegel, M.D., Senior Investigator, MDB, NIADDKD
Andrew W. Saxe, M.D., Surgery Branch, NCI
Leonard Deftos, M.D., University of California, San Diego
Dr. J. Heersche, St. Michael's Hospital, Toronto
R. W. Downs, Jr., M.D., Senior Clinical Associate, MDB, NIADDKD
S. J. Marx, M.D., Senior Investigator, MDB, NIADDKD
G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDKD

COOPERATING UNITS (if any)

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK , NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.75	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hypercalcemia is a common and serious complication of cancer. In some patients, hypercalcemia occurs without direct tumor invasion of bone, presumably via a humoral mechanism. The identity of humoral factor(s) is not known, although PTH or prostaglandins may be the cause in some cases. The project goal is to identify factors responsible for humoral hypercalcemia of malignancy. This involves study of patients with this syndrome as well as an animal model for the syndrome, a Leydig cell tumor of Fischer rats.

Objective: To identify the factor(s) responsible for humoral hypercalcemia in Fischer rats bearing a transplantable Leydig cell tumor, and in humans with humoral hypercalcemia of malignancy.

Methods employed: The Rice H-500 Leydig cell tumor was obtained from the Mason Tumor Bank in Worcester, Massachusetts. Fischer 344 rats are injected subcutaneously with minced tumor and then studied (as the tumor grows) by obtaining blood and urine samples for measurement of indices of mineral metabolism including: serum calcium, phosphorus, creatinine, and parathyroid hormone, and urinary cAMP. Bones from tumor-bearing and control animals are obtained for histomorphometric studies. The tumor has also been established in tissue culture for studies of secreted factor(s) in the medium. Conditioned medium can be assayed for bone-resorbing factors in a variety of target cell systems.

Blood and urine samples from patients with humoral hypercalcemia of malignancy have also been obtained for measurement of indices of mineral metabolism.

Major findings: We have confirmed the original observations of Rice that the Leydig cell tumor represents a model of humoral hypercalcemia. No evidence of bone metastasis is present and hypercalcemia is corrected by tumor resection. We have extended these observations by showing that PTH, measured by a sensitive radioimmunoassay specific for the aminoterminal portion of rat PTH, is suppressed in hypercalcemic tumor-bearing rats. This result provides strong evidence that tumor-provoked hypercalcemia is due neither to ectopic PTH secretion by the tumor nor to a parathyrotrophic factor secreted by the tumor. There is no detectable PTH in the culture medium of tumor cells. We have also measured urinary cAMP excretion, an indirect index of PTH action, and found that U_{cAMP} is increased in tumor-bearing hypercalcemic rats when expressed as a function of creatinine clearance. If confirmed, this result suggests that the tumor may secrete a "PTH-like" factor, capable of causing osteolysis and activating renal adenylate cyclase. These findings are analogous to certain patients with humoral hypercalcemia due to malignancy (e.g. squamous lung carcinoma) who excrete increased amounts of nephrogenous cAMP although PTH secretion is suppressed.

Potent bone-resorbing activity has been found in the medium from cultured tumor cells.

Significance to Biomedical Research and Program of the Institute: These studies have important implications for our understanding of normal and abnormal calcium homeostasis and for our ability to treat hypercalcemia complicating cancer. Identification of a humoral factor should be more readily achieved in the rat model and information gained therefrom can then be applied to the human syndrome.

Proposed Course of Project: The future course of the project involves assay of media from cultured cells, tumor extracts, and serum samples in a variety of assays for several properties (e.g. bone resorption, renal cell cAMP) in an effort to identify the factor responsible for hypercalcemia. Biosynthetic studies of cultured tumor cells will also be performed. Identification of a factor(s) should permit testing of sera and extracts from patients with humoral hypercalcemia.

Publications: None

ANNUAL REPORT - 1980-1981

CLINICAL ENDOCRINOLOGY BRANCH

Dr. Weintraub was the recipient of the Ernst Oppenheimer award of the Endocrine Society. This award is presented annually to an outstanding young scientist for contributions to endocrine research. Dr. Robbins was awarded the Parke-Davis Lectureship of the American Thyroid Association. He was the second recipient of this honor, which is given to a senior scientist in recognition of contributions to thyroidology and thyroid research. Dr. Robbins also was Visiting Professor and recipient of the Samuel Haines Lectureship at the Mayo Clinic, an award honoring Dr. Haines, who was a pioneer in the study of thyroid diseases.

Visiting scientists from laboratories in France, India, Italy and South Africa participated in the work of the Branch. Two senior scientists of the Branch - Drs. Cahnmann and Edelhoch - spent a number of weeks in France and Italy where they took part in collaborative research with laboratories in Paris, Marseilles and Naples.

I. Thyroid Biochemistry

A. Thyroid Cell Membrane and the Mechanism of TSH Action

The correlation of TSH binding to thyroid cell membrane with the activation of adenylate cyclate has been examined for TSH derived from various sources. These include human serum, the serum of mice bearing thyrotropic tumors, the culture medium in which these tumors were grown and various subfractions of bovine pituitary glands. Each source contains TSH subfractions which can be separated by gel filtration and which probably differ in carbohydrate residues. In human serum, a large molecular weight form appears to be bound to a protein component of serum.

Certain of the TSH forms have a high ratio of receptor binding to cyclase activation, and they appear to act as competitive inhibitors of bovine TSH action. It appears likely that differences in carbohydrate content are responsible for this dichotomy and it is evident that the degree of receptor binding is not directly related to biological activity of TSH (Weintraub, Joshi).

Further characterization of solubilized adenylate cyclase has been accomplished. The activation or lack of activation of the enzyme by various guanine nucleotide analogs differed with the substrate employed, being more evident with App(NH)p than with ATP. Fluoride, which was used in these experiments, was shown to inhibit GTPase activities in the thyroid cell membrane, possibly accounting for the synergistic behavior of fluoride and GTP. Kinetic analysis of activators and inhibitors indicated that there either are two independent guanine nucleotide-binding sites, or two sites exhibiting negative cooperativity. Such analysis also revealed that inhibition of GDPβS could not be explained by simple competitive inhibition. (Wolff, Goldhammer)

Previous work on the role of adenosine in steroid hormone secretion has now been extended to the guinea pig thyroid. Adenosine analogues stimulated cyclic AMP accumulation, but were only partial agonists. The pattern of response resembled that seen earlier in the adrenal gland. (Wolff, Fradkin)

B. Thyroid Hormone Secretion

Interest in the role of tubulin in secretion has led to extensive studies of this ubiquitous protein. Previous work which demonstrated that lactoperoxidase binds to tubulin has permitted a rapid purification method which makes use of affinity chromatoraphy employing lactoperoxidase bound to a matrix. Tubulin which is 90% pure can be obtained within 2 to 3 hours without exposure to glycerol or GTP. Compared to standard preprations of tubulin, the more highly purified material is free of high molecular weight microtubule-associated proteins, and has a higher critical concentration for polymerization. (Wolff, Rousset)

Antibodies to colchicine have been produced in rabbits and were compared to tubulin in terms of colchicine binding. The affinity of the antibody was high enough to prevent colchicine fluorescence in the presence of tubulin and to restore polymerization in colchicine-inhibited tubulin preparations. The chemical specificity of the antibody-binding site contrasted with that of tubulin, in that it tolerated greater variation in the tropolone moiety.

A detailed study is currently under way to evaluate the role of calcium in tubulin polymerization. It has been found that factors which promote nucleation (such as increased temperature, microtubule seeds or 16S oligomers) decrease the intrinsic high Ca^{2+} sensitivity for inhibition of polymerization, and that calmodulin enhances the sensitivity. (Wolff, Berkowitz, Lee)

C. Thyroid Hormone Synthesis

The chemical mechanism of the synthesis of thyroxine within the polypeptide chain of thyroglobulin has been further clarified by comparing non-enzymatic iodination (I_3^- or ICl) with enzymatic iodination employing lactoperoxidase. Since both methods lead to the production of one dehydroalanine residue for each molecule of thyroxine formed, the reaction mechanisms are probably similar. It is suggested that the iodination of two appropriately located tyrosine residues is followed by oxidation of one of these to a phenoxy cation, leading to a charge transfer complex and formation of a transient quinol ether and an eventual covalent diphenyl ether bond. A pH above neutrality is required for coupling. (Cahnmann, Gavaret)

D. Thyroxine-Protein Interactions

Previous work with thyroxine-binding globulin (TBG) revealed a metastable state for this major transport protein as it exists in plasma. It has now been shown that the presence of a hormone molecule in the binding site increases resistance of TBG to denaturation by mild acid (pH 4.4), low concentrations of guanidinium chloride (1.75 M) or heat. This stabilization is accompanied by an allosteric change in which TBG becomes more compact, approaching a spherical shape. Tryptophanyl spectroscopic analysis demonstrated that the four Trp residues are heterogeneous, and that one of the surface residues moves toward the interior of the molecule when T_4 is bound. These effects of hormone binding on protein structure can serve as a model for the interaction of thyroid hormone with biologically active receptors. (Robbins, Edelhoch, Grimaldi)

The controlled photolysis of thyroxine and its analogs was investigated preliminary to the use of this procedure for photoaffinity labeling of T_4- and T_3-binding proteins. Using long-wave ultraviolet light (above 330 nm) and a relatively non-polar solvent (methanol), conditions were found to give a 46% yield of T_3 from T_4 and no damage to the diphenyl ether bond. Similar monodeiodination of the phenolic ring was found with several T_4 analogs. This procedure is suitable for the preparation of specifically labelled metabolites of T_4 which are not easily obtainable by chemical synthesis. (Cahnmann and van der Walt)

E. Thyroid Hormone-Cell Interactions

In previous work, rhodamine-labeled triiodothyronine was shown by video-intensification fluorescence microscopy to enter cultured fibroblasts by receptor-mediated binding to coated pits followed by endocytosis. The present study used monolayer cultures of monkey hepatocarcinoma cells and nuclear uptake of ^{125}I-labeled T_3 to show that the fluorescent derivatives reached the same nuclear receptors as did the hormone. The affinities of the derivatives, however, were about 100 times lower than that of the parent hormone. (Cheng, Robbins, Johnson)

F. Thyroid Hormone Metabolism

Fasting and refeeding have profound effects on the metabolism of thyroid hormones in man and other animals, such that fasting enhances the conversion of T_4 to T_3 by monodeiodination. To investigate the mechanism of this effect, T_4 metabolism was studied in primary monolayer cultures of rat hepatocytes. T_4 metabolism in these normal hepatocytes, unlike previous findings in monkey hepatocarcinoma cells, was more physiological, showing equal deiodination of the inner and outer rings as well as the production of glucuronoconjugates. Incubation with combinations of glucose, glucagon and insulin revealed that insulin stimulated 5'-deiodination whereas glucose had no effect. Glucagon inhibited the change produced by insulin but had no effect in the absence of insulin. These findings suggest that the effects of feeding in the whole animal are related to changes in hormones rather than in glucose per se. (Robbins, Sato)

G. Thyroid Hormone Action

Quantitative changes in nuclear proteins from the livers of control, thyroidectomized and T_3-treated rats were measured by computer analysis of two-dimensional gel electrophoresis patterns. Approximately 500 protein subunits could be distinguished. Of these, 102 decreased markedly or disappeared after thyroidectomy. Within 6 hours after giving T_3, 13 proteins reappeared, and 67 additional proteins reappeared by 24 hours. Two proteins not found in normal liver were seen.

Acetylation of nuclear histones and low molecular weight proteins was depressed after thyroidectomy and was increased above control after T_3 administration. Incorporation of phosphate from ATP, occurring in proteins which ranged in size up to 100,000 molecular weight, was decreased in most of them after thyroidectomy, and also was increased by T_3, especially in those over 68,000 Daltons. Incorporation of ADP ribose from NAD also was depressed after thyroidectomy and decreased further after T_3 in most of these, but increased in a few. It is suggested that the increase in protein synthetic rate caused by T_3 may be the result of increased acetylation and phosphorylation of histones and other nuclear proteins which accelerate transcription of already active genes.

Since transcriptionally active genes are preferentially digested by micrococcal nuclease, the digestion of DNA from thyroidectomized and T_3-treated rats was measured. Preliminary data showed an increased rate of DNA splitting after T_3 administration. Digestion with DNase I gave similar results. (Rall, Nikodem)

H. Studies of Thyroid Disease

The exposure of the thyroid gland to high dose therapeutic radiation is known to cause thyroid cell death, but few cases of nodule formation have been reported. Six such cases were observed after radiation exposure, usually for Hodgkins Disease, of 2500 to 4000 rads; three had thyroid carcinoma. Of special interest was the finding of severe, diffuse cellular atypia in aspiration biopsies and in the surgical specimens from four cases. In two, this could be ascribed to radiation effects on the thyroid epithelial cells. It is apparent that radiation-induced thyroid neoplasia may be more frequent after high dose radiation than previously appreciated. (Robbins, Pretorius)

Evaluation of serum thyroglobulin (TG) measurement in the management of differentiated thyroid cancer was accomplished in 49 patients. Serum TG, when detectable, paralleled changes in serum TSH. After stopping T_3 for 2 weeks in previously thyroidectomized patients, serum TG was undetectable in most patients with negative ^{131}I scans and was above 10 ng/ml in all patients with definite metastases. However, a high serum TG did not distinguish metastases from residual normal thyroid. Since serum TG and ^{131}I scans were not always concordant, TG measurement, while useful in follow-up care of thyroid cancer, cannot replace ^{131}I testing. (Robbins, Goldman)

The utility of low dose (30 mCi) ^{131}I for thyroid remnant ablation is under study as an alternative to the usual 75 to 100 mCi dose. Preliminary results suggest that this therapy may be useful, especially in young patients, to reduce whole body radiation exposure. (Robbins, Pretorius)

T_3 transport and nuclear binding in skin fibroblasts of 4 patients with peripheral thyroid hormone resistance has been compared with 8 normal subjects. No significant differences were found in mean nuclear binding capacity (~ 4000 sites/cell) or apparent dissociation constant (~ 10^{-10} M). Since preliminary results indicate that fibroblasts from affected subjects show biochemical resistance to thyroid hormone, it appears that the defect is not in nuclear T_3 receptors or in cellular or nuclear transport of the hormone. (Weintraub, Fein)

Further study of the syndrome of postpartum thyroiditis with transient hyper- and hypothyroidism has shown that thyroid dysfunction correlates well with changes in antithyroid antibody titers. Preliminary data also suggest that there is a possible defect in suppressor lymphocyte function as well as a possible association with a D locus antigen of the HLA system. (Weintraub, Fein)

The association of thyroid dysfunction with Sjogren's syndrome and systemic lupus erythematosus (SLE) has been recognized, but the frequency is unknown. In a study of 14 patients with Sjogren's syndrome, 64% had decreased thyroid reserve or frank hypothyroidism. Data are now being collected in SLE. (Weintraub)

The randomized study of cytopheresis and plasmapheresis in the treatment of Graves' ophthalmopathy has been completed in 7 patients. Preliminary findings suggest that some patients have had moderate subjective and objective improvement, in conjunction with a decrease in thyroid stimulating immunoglobulin. (Weintraub, Magner)

II. Polypeptide Hormones

A. Biosynthesis of Glycoprotein Hormones

The interrelation between peptide chain synthesis, glycosylation, subunit combination and secretion of the thyroid stimulating hormone has been further elucidated. These studies have been done with primary cultures of mouse thyrotropic tumor cells. Subcellular fractions enriched in rough and smooth endoplasmic reticulum and golgi components were obtained by density gradient centrifugation and nascent chains were solubilized by ^{3}H-puromycin displacement or treatment with detergent. Nascent chains displaced by puromycin showed a predominance of unglycosylated subunits. The size distribution suggested a "translational block" or pause in chain elongation at the time of dolichol addition, which favored the accumulation of chains of particular molecular weights. A time-dependent progression of 11 K to 18 K α subunits occurred in the rough endoplasic reticulum, while most of the α- and β-subunit combination forming the 21 K moiety took place in the smooth reticulum. Incubation of cells with tunicamycin, an inhibitor of glycosylation, gave rise to non-glycosylated TSH, half of which were degraded intracellularly before secretion. The data are consistent with previous indications that glycosylation is required for subunit combination but not for secretion from the cell. (Weintraub, Magner)

The biosynthesis of human chorionic gonadotrpoin has been studied in a continuous culture of choriocarcinoma cells. Incorporation of ^{3}H-fucose into the intracellular α-subunit (common to all the glycoprotein hormones) was unequivocally demonstrated. This was unexpected since α-subunit isolated from pregnancy urine does not contain fucose. As is the case with TSH, enzymatically deglycosylated α-and β-subunits of hCG show inhibition of combining properties. These studies are being extended to an investigation of the properties of ectopic α- and β- subunits - i.e., subunits secreted by chorionic and other neoplasms. Some iso-β-subunits combine well with normal hCG-α whereas others do not. The cause for these differences is being sought. (Rosen, Weintraub, Morrow, Jones-Brown, Fein)

B. Ectopic Polypeptide Hormone Syndromes

The occurrence of the α- and β-subunits of chorionic gonadotropin was investigated in the serum of patients with primary hyperparathyroidism. Two patients with parathyroid carcinoma had elevated levels of both subunits. One of these tumors contained high subunit concentrations, in contrast to their absence or much lower concentration in hyperplastic parathyroid tissue. Analysis of blood containing high parathyroid hormone levels obtained by venous cathetrization showed a mild elevation of CG-α in only one of 42 patients with parathyroid hyperplasia or adenoma. In 30 patients with multiple endocrine neoplasia type I, 8 had elevated CG-α in conjunction with a pancreatic islet cell tumor. Thus, screening of hyperparathyroid patients for CG-α may be used to detect parathyroid carcinoma or associated islet cell tumors.

In 32 patients with Zollinger-Ellison syndrome (gastrin-secreting tumors), CG-α was elevated in 15 and CG-β in 4. Most of these elevations occurred in patients with malignant gastrinoma, and the development of an elevation during follow-up of patients thought to have benign gastrinoma is suggestive of malignancy. (Rosen, Weintraub, Morrow)

An evaluation of the measurement of pregnancy-specific beta$_1$-glycoprotein (SP_1) has been made in patients with fibrosarcoma and other sarcomas, since fibroblast cell cultures have been found to secrete this protein. The clinical studies, however, failed to indicate a utility as a tumor marker. The synthesis of SP_1 was studied in cultured human placental cells transformed by simian virus (SV40). The temperature sensitive mutant (tsA) showed marked enhancement of SP_1 production at the restrictive temperature. SP_1 secreted by these cells could be induced by sodium butyrate. Its molecular properties were similar to those of SP_1 purified from normal placenta, and it appears to consist of various aggregates of a 43,600 molecular weight unit. (Rosen)

The presence of human placental lactogen was examined in endometrial biopsies by an immunoperoxidase histochemical technique. Positive cells were seen in association with intrauterine pregnancy but not in tubal pregnancy, pseudodecidua in progestrogen-treated patients or other nonpregnant endometrial tissue. This procedure may be useful as a marker for intrauterine pregnancy. (Rosen)

C. Gonadotropin Pathophysiology

In the continued investigation of a family with hypogonadotropic hypogonadism with anosmia (Kallman's syndrome) old church records have disclosed a consanguineous relationship six generations earlier. Two new cases have been discovered and further evidence of heterogeneity has been obtained.

Further study of a patient with hyperpigmentation, skin thickening, hyperprolactinemia with galactorrhea, splenomegaly and monoclonal IgG kappa light chain indicates that she probably can be included in the so-called POEMS syndrome although she lacks the feature of polyneuritis. Studies with her serum IgG failed to augment melanin secretion *in vitro* or to simulate prolactin secretion by rat pituitary tumor. (Rosen, Morrow)

III. Studies on Protein Structure and the Interaction of Proteins with Cell Membranes

A more rapid procedure for purifying coated vesicles from bovine brain has been developed. These vesicles dissociate into uncoated vesicles and clathrin when the pH is raised from 6.5 to 7.5, and they reform when the pH is lowered. This has permitted isolation of the membranes associated with coated vesicles, and these are now under study.

Polymerization of clathrin to form coat structures (devoid of membranes) was found to be accelerated by antimalarial drugs and by phenothiozines. Since many of these compounds inhibit receptor-mediated endocytosis in intact cells, it was suggested that they may act by regulating clathrin function in the coated pits. (Edelhoch, Nandi, Pretorius, van Jaarsveld)

Further insight on the subunit structure of thyroglobulin was obtained by the finding that a protease which is a minor contaminant of horseradish peroxidase can result in the formation of one of the major subunit components found in thyroglobulin preparations. It was concluded that the 300,000 molecular weight subunit is probably the only chain synthesized by the thyroid gland, and the second component is produced by proteolytic digestion. (Edelhoch, van Jaarsveld)

IV. Mechanism of Hormone Action

Previous studies showed that the soluble adenylate cyclase produced by *Bordetella pertussis* is activated by calmodulin, which was identified as a component of the organism or the culture medium. Since the ability to respond to calmodulin is lost when the bacterial cyclase is purified, a search for an additional factor in the interaction was undertaken. It was found that various amphophiles such as phospholipids activated the cyclase 3-10 fold, and that gangliosides are inhibitory. Ca^{++} (in the absence of calmodulin) is required. The amphophiles also sensitize the adenylate cyclase to Ca^{2+}/calmodulin and are synergistic with calmodulin. The amphiphiles act by altering the Vmax of the enzyme and not the Km.

In the course of these studies, it was found that a large variety of protein and tissue preparations contain a protein activator of *B. pertussis* adenylate cyclase. It was shown that this results from the presence of calmodulin in these protein preparations. The high sensitivity of the bacterial cyclase makes it an ideal system for the detection of trace amounts of calmodulin. (Wolff, Goldhammer, Kilhoffer)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45000-14 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Thyroxine Protein Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
	H. Edelhoch	Chief, Prot. Struct. Sec.	CEB NIADDK
Other:	S. Grimaldi	Visiting Fellow	CEB NIADDK
	M. Phyillaier	Biologist	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.9	PROFESSIONAL: 1.5	OTHER: .4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of thyroxine (T4) on several molecular properties of thyroxine-binding globulin (TBG) have been evaluated. Changes in sedimentation constant and relaxation time indicate that TBG becomes more compact when T_4 is bound, and this is associated with increased resistance to denaturation by acid or guanidinium chloride. T_4 binding also alters the emission and excitation spectra of TBG, reflecting differing environments of its 4 tryptophanyl residues. These studies provide a model for allosteric effects of thyroid hormone binding on receptor proteins.

PHS-6040
(Rev. 2-81)

Project Description:

Thyroxine binding globulin (TBG), the major transport protein for thyroid hormones in human serum, was shown in earlier work to be denatured irreversibly under mild conditions. T_4 binding is lost, but the secondary and tertiary structure changes only slightly. We now have investigated the influence of occupancy of the hormone binding site on TBG stability. Using tryptophanyl fluorescence, we have shown that $TBG \cdot T_4$, in contrast to unliganded TBG, is more resistant to denaturation by mild acid (pH 4.4) and by low concentration guanidinium chloride (1.75 M). This stabilization is accompanied by an altered conformation - i.e., an allosteric effect - resulting from T_4 binding. Sedimentation velocity analysis and polarization of dansyl fluorescence show that $TBG \cdot T_4$ is a more compact molecule than native TBG, and its shape approaches that of a sphere.

Further studies on TBG structure utilizing tryptophan fluorescence spectroscopy demonstrated a heterogeneity of the four tryptophanyl residues of the protein. The quenching of Trp fluorescence which is seen when T_4 is bound involves one or more Trp residues which are in a nonpolar environment - i.e., interior. The collisional quencher, acrylamide, on the other hand, affects primarily one or more Trp residues on the protein surface. The wavelength of maximum acrylamide quenching (345 nm) is shifted to shorter wavelength when T_4 is bound, owing to a change in protein conformation.

It is postulated that the conformational change of TBG induced by hormone binding is responsible for the increased stability, and these studies provide a model for the interaction of thyroid hormones with biologically active receptors.

Publications:

1. Erard, F., Cheng, S.-Y., and Robbins, J.: Affinity labeling of human serum thyroxine-binding globulin with N-bromoacetyl-L-thyroxine: Identification of the labeled amino acid residues. Arch. Biochem. Biophys. 206: 15-20, 1981.

2. Cheng, S.-Y., Rakhit, G., Erard, F., Robbins, J., and Chignell, C. F.: A spin label study of the thyroid hormone-binding sites in human plasma thyroxine transport proteins. J. Biol. Chem. 256: 831-836, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45003-07 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Synthesis of Thyroid Hormone Residues in Thyroglobulin (formerly Model Reactions for the Synthesis of Thyroxine Residues in Thyroglobulin)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. J. Cahnmann	Scientist Emeritus	CEB NIADDK
Other:	J.-M. Gavaret	Charge de Recherche	Coop Unit (see below)
	J. Nunez	Chief	Coop Unit (see below)

COOPERATING UNITS (if any)
Unitè de Recherche sur la Glande Thyroide et la Regulation Hormonale, INSERM, Bicetre, France

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .5	PROFESSIONAL: .4	OTHER: .1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

No matter which method of iodination (enzymatic or nonenzymatic) is used for the synthesis of thyroid hormone residues in thyroglobulin, the formation of each hormone residue is always accompanied by that of one dehydroalanine residue. Thyroglobulin can be iodinated with iodine monochloride at pH 5.5, but the formation of hormone and dehydroalanine residues takes place only above neutrality.

PHS-6040
(Rev. 2-81)

Project Description:

We reported last year that, in the thyroid peroxidase-catalyzed synthesis of thyroid hormone residues in thyroglobulin, one dehydroalanine residue is produced for each hormone residue formed. We have now studied the nonenzymatic as well as lactoperoxidase-catalyzed synthesis of hormone residues. Iodine-free [U-^{14}C]tyrosine-labeled thyroglobulin was iodinated in the presence of lactoperoxidase and glucose-glucose oxidase or with I_3^- or I^--chloramine T or ICl. In all instances, the synthesis of each hormone residue was accompanied by that of one dehydroalanine residue suggesting that similar reaction mechanisms prevail in enzymatic and nonenzymatic hormone synthesis. This prompted us to propose the following model for the hormone synthesis in thyroglobulin: Two tyrosine residues appropriately located for coupling are iodinated in an ionic iodination reaction or in one involving free radicals (enzymatic iodination). Then, one of the iodotyrosine residues is oxidized to a phenoxy cation (in an ionic reaction or in two one-electron transfer steps). This results in the formation of a charge transfer complex between the two interacting iodotyrosyl residues (which is a zwitterion-biradical resonance hybrid). Establishment of a covalent bond between the two residues leads to a quinol ether residue (of possibly only fleeting existence) whose breakdown leads to one hormone residue and one dehydroalanine residue.

While thyroglobulin is not iodinated with I_3^- or with I^--chloramine T at an acid pH, ICl is a good iodinating agent at pH 5.5. However, no coupling occurs at that pH although hormonogenic iodotyrosyl residues have been formed. A pH above neutrality is required for coupling.

Publications:

1. Gavaret, J.-M., Cahnmann, H. J. and Nunez, J.: The fate of the "lost side chain" during thyroid hormonogenesis. J. Biol. Chem. 254: 11218-11222, 1979.

2. Gavaret, J.-M., Nunez, J., and Cahnmann, H. J.: Formation of dehydroalanine residues during thyroid hormone synthesis in thyroglobulin. J. Biol. Chem. 255: 5281-5285, 1980.

3. Gavaret, J.-M., Cahnmann, H. J., and Nunez, J.: Thyroid hormone synthesis of thyroglobulin. The mechanism of coupling reaction. J. Biol. Chem. 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45004-10 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Structure of Polypeptide and Protein Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Edelhoch	Senior Scientist	CEB NIADDK
	H. T. Pretorius	Staff Fellow	CEB NIADDK
	P. Nandi	Visiting Scientist	CEB NIADDK
	P. van Jaarsveld	Visiting Scientist	CEB NIADDK
	G. Irace	Visiting Associate	CEB NIADDK
Other:	R. E. Lippoldt	Sr. Health Services Officer	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.9	PROFESSIONAL: 3.7	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have prepared coated vesicles from bovine brain by a modified procedure. The dissociation of coat and vesicles and the reversal have been accomplished.

Two groups of pharmacologic agents, antimalarials and phenothiazines, markedly stimulate the polymerization of clathrin to coat structures. They have also been shown by others to inhibit endocytosis.

PHS-6040
(Rev. 2-81)

Project Description:

The work is continuing on the role of clathrin and coated vesicles in receptor-mediated endocytosis. This mechanism of internalization of proteins and hormones is proving to be very common and involves a wide variety of metabolic species.

An abbreviated procedure to isolate highly purified coated vesicles in good yield has been developed. It has been shown that coated vesicles dissociate into uncoated vesicles and clathrin when the pH is raised from 6.5 to 7.5 and are reformed when the pH is returned to 6.5. The uncoated vesicles have been separated from clathrin on sucrose gradients. We are looking at the properties of these vesicles as a natural source of bilayer material for other types of studies.

Two groups of pharmacologic agents, i.e. antimalarials and phenothiazines, have been shown to accelerate the polymerization of clathrin to form coat structures (devoid of membrane). Many of these compounds have also been shown to inhibit receptor-mediated endocytosis in various types of cells. It is, therefore, possible that some of these drugs are active by virtue of their interaction with clathrin in the coated pits or vesicles and thereby regulate cellular machinery involved in endocytosis.

A smaller fraction of our work continues on the characterization of the subunit structure of thyroglobulin. We have been able to convert one of the two principal subunits to the other by a preparation of horseradish peroxidase. The enzyme responsible, however, is a minor contaminant present in our commercial enzyme preparation. We have, therefore, concluded that only one chain of ~ 300,000 is synthesized by the thyroid gland and the second chain is produced by proteolytic digestion.

Publications:

1. Nandi, P. K., Pretorius, H. T., Lippoldt, R. E., Johnson, M. L., and Edelhoch H.: Molecular properties of the reassembled coat protein of coated vesicles. Biochemistry 19: 5917-5921, 1980.

2. van Jaarsveld, P. P., van der Merwe, J., van der Walt, B., and Edelhoch, H.: Enzymatic conversion of the major polypeptide chains of thyroglobulin. Endocrinology 108: 1285-1292, 1981.

3. Edelhoch, H., Cheng, S.-Y., and Irace, G.: Luminescences from biological and synthetic macromolecules: Spectroscopic properties of Rhodamine B-labeled thyroid hormone. New York Acad. Sci., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM-45009-14 CEB

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies in Thyroid Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
	B. D. Weintraub	Medical Director	CEB NIADDK
	H. T. Pretorius	Staff Fellow	CEB NIADDK
	J. Magner	Clinical Associate	CEB NIADDK
	J. Fradkin	Clinical Associate	CEB NIADDK
Other:	E. W. Chu	Head, Cytopath. Sec.	LP NCI
	M. Brennan	Head, Surg. Metab. Sec.	SB NCI
	B. Line	Staff Physician, Dept. of Nuc. Med.	CC NCI
	C. Ramacciotti	Guest Worker	CEB NIADDK
	T. J. Kinsella	Sr. Investigator	ROB NCI
	S. H. Barsky	Expert	LP NCI
	A. Steinberg	Medical Director	ARB NIADDK
	C. Eil	Staff Endocrinologist	Naval Med. Ctr.

COOPERATING UNITS (if any)

National Cancer Institute; Clinical Center; National Naval Medical Center, Bethesda

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.3	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the diagnosis of thyroid nodules and goiter, we studied 6 patients who developed thyroid nodules, 3 with malignancy, after high dose radiation exposure (> 2500 r). Previously, such exposure was thought to cause only thyroid failure. In the treatment of thyroid cancer, serum thyroglobulin (TG) measurement was compared with total body ^{125}I scanning in 49 patients. Screening for residual thyroid tissue or metastases by serum TG requires discontinuation of thyroid hormone replacement. A combination of TG level and ^{131}I scanning is preferable to either test alone. In the syndrome of inappropriate TSH secretion, nuclear T_3 binding in fibroblasts cultured from 4 patients was examined. No difference from normal was found in the number or affinity of nuclear receptors. In studies on lymphocytic thyroiditis, association with the postpartum state, with Sjogren's syndrome and with systemic lupus erythematosus has been studied. Completion of detailed examinations on 14 patients with Sjogren's syndrome showed that 64% had compensated or frank hypothroidism.

PHS-6040
(Rev. 2-81)

Project Description:

1. Diagnosis of Thyroid Nodules and Goiter

From 1974 to 1980, we encountered six patients with thyroid nodules after high dose radiotherapy (2500 to 4000 rad to the thyroid), usually for Hodgkin's disease. Fine needle aspiration cytology showed moderate to severe cellular atypia in four; in two this could be ascribed to radiation effect, thus complicating the utility of aspiration cytology in such patients. In one additional patient, the cellular atypia was related to lymphocytic thyroiditis, and in only one, to carcinoma. Of the five patients who had thyroidectomies, however, three had carcinomas. Two patients had lymphocytic thyroiditis, and four had compensated or overt hypothyroidism. Although thyroid failure has been recognized as a common sequela of such radiation exposure, our findings indicate that thyroid nodules and carcinoma may be more frequent than previously appreciated.

2. Treatment of Thyroid Cancer

Evaluation of serum thyroglobulin (TG) measurement in the management of functioning thyroid cancer has been completed in 49 patients who had simultaneous radioiodine whole body scans. In thyroidectomized patients with thyroid remnants or metastatic cancer, serum TG paralleled changes in serum TSH, and was usually low or undetectable when TSH was suppressed. After stopping T_3 therapy for 2 weeks, serum TG was < 2 ng/ml in most patients with no detectable ^{131}I uptake and was > 10 ng/ml in all patients with definite metastases. However, a high serum TG level did not distinguish metastases from residual normal thyroid tissue. Serum TG was elevated in some patients with negative scans (13%) and was undetectable in some patients with positive scans (44%). Although serum Tg is useful in the follow-up care of thyroid cancer, it cannot replace ^{131}I scanning.

The utility of low dose (30 mCi) ^{131}I for thyroid remnant ablation has been evaluated in 16 patients. In 7 (44%), a negative thyroid scan was achieved after a single dose. While repeated 30 mCi doses (at 6 month intervals) further decreased neck uptake in most of the remaining cases, total ablation usually required 3 or more doses. Our experience suggests that the use of 30 mCi ^{131}I ablative therapy as the initial dose is worthwhile, but the advantage of repeated small doses is uncertain.

3. Inappropriate Secretion of TSH

We have studied T_3 transport and nuclear binding in skin fibroblasts from 8 normal subjects and 4 patients with peripheral thyroid hormone resistance, one subclass of inappropriate secretion of TSH. Skin fibroblasts were grown in serial passage, dispersed with mild trypsin digestion and incubated at 37°C in serum-free medium with various concentrations of ^{125}I-T_3 in the presence or absence of unlabeled T_3. After equilibrium had been reached (45-60 min), cells were collected and nuclei isolated and counted for radioactivity. In normal fibroblasts nuclear binding of $^{125}I-T_3$ was saturable and showed a single class of high affinity receptors, with a mean binding capacity (Ro) of 3800 ± 500 nuclear sites/cell and mean apparent dissociation constant (Kd) of $1.0 \pm 0.10 \times 10^{-10}$ M. Competition of nuclear $^{125}I-T_3$ binding by thyroid hormone analogues showed affinity characteristics similar to those reported for other tissues. Fibroblasts from 4 patients with thyroid hormone resistance were analyzed at multiple passages and showed no significant differences in nuclear $^{125}I-T_3$ binding (Ro = 4890 ± 460 sites/cell; Kd = $1.04 \pm 0.07 \times 10^{-10}$ M).

Since preliminary studies suggest that skin fibroblasts from the patients manifest the biochemical resistance to thyroid hormone, these data indicate that the defect is not in nuclear T_3 receptors or in cellular or nuclear transport mechanisms for the hormone. The site of the defect is currently under investigation.

4. Postpartum Thyroiditis with Transient Hypothyroidism

We are currently following at least 9 women with well documented postpartum lymphocytic thyroiditis associated with transient hyperthyroidism followed by transient hypothyroidism. In general, the thyroid dysfunction correlates with a rise in serum anti-thyroglobulin as well as antimicrosomal antibodies. However, we have now encountered one patient who only manifested the transient hyperthyroid phase at 2-3 months postpartum, but never manifested the later hypothyroid phase; interestingly, she is the only patient who has not shown any detectable antithyroid antibodies (both titers < 100). To gain further insight into the basic immunologic mechanisms involved in this disease, we have begun to measure a variety of parameters of lymphocyte function during and after pregnancy, in both controls and patients who have manifested the syndrome in prior pregnancies. Preliminary results suggest a possible defect in suppressor cell function as well as a possible association of this disease with a D locus antigen of the HLA system.

5. Thyroid Dysfunction in Sjogren's Syndrome and Systemic Lupus Erythematosys

Although it has been recognized that Sjogren's syndrome (S.S.) and systemic lupus erythematosys (S.L.E.) may be associated with thyroid dysfunction, the prevalence of this association is not known with certainty. We have studied this association using sensitive tests of thyroid function including multiple measurements of T_3, T_4, free T_4, TSH, antithyroid antibodies as well as TRH tests and thyroid aspiration biopsies. Of 24 patients with S.S., 10 had been treated with thyroxine by other physicians and were not re-evaluated except for anti-thyroid antibodies, which were elevated in 4 of the 4 tested. Of the 14 remaining patients that we evaluated, 9 showed at least one abnormality of thyroid function (usually increased basal or TRH-stimulated TSH and anti-thyroid antibodies); 2 of these patients had decreased T_4 and free T_4 as well as clinical hypothyroidism requiring treatment with T_4. Thus, of the 14 patients that we evaluated, 64% had decreased thyroid reserve or frank hypothyroidism; of the original group of 24, as many as 79% may have had thyroid dysfunction.

Results in the patients with S.L.E. are more preliminary but also show a high prevalence of thyroid dysfunction. Interestingly, certain of the S.L.E. patients have had well-documented hyperthyroidism (presumably caused by Graves' disease), which was not observed in the patients with S.S.

Publications:

1. Fein, H. G., Goldman, J. M., and Weintraub, B. D.: Postpartum lymphocytic thyroiditis in American women: A spectrum of thyroid dysfunction. Amer. J. Obstet. Gyn. 138: 504-510, 1980.

2. Karsh, J., Pavlidis, N., Weintraub, B. D., and Moutsopoulos, H. M.: Thyroid disease in Sjogren's syndrome. Arth. Rheum. 23: 1326-1329, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45010-11 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Ectopic Protein and Subunit Production by Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. W. Rosen	Medical Director	CEB NIADDK
	B. D. Weintraub	Medical Director	CEB NIADDK
	J. S. Morrow	Clinical Associate	CEB NIADDK
Other:	S. Aaronson	Lab Chief	LCMB NCI
	J. Osborne	Sr. Investigator	IR MD NHLBI
	E. Heyderman	Sr. Lecturer, Dept. Path.	St. Thomas's Hosp., London
	D. Tormey	Assoc. Prof., Dept. Med.	U. Wisc. Med. Ctr.
	C. Horne	Prof., Dept. Path.	U. Aberdeen, Scotland
	D. Zopf	Sr. Investigator	LP NCI
	J. Chou	Sr. Investigator	PRB NICHD
	R. A. Kyle	Staff Physician	Mayo Clinic
	D. McCarthy	Sr. Investigator	DDB NIADDK
	J. Stock	Clinical Associate	MDB NIADDK

COOPERATING UNITS (if any)

NCI; St. Thomas's Hosp., London, England; U. of Aberdeen, Scotland; U. Wisc. Med. Ctr.; NICHD; NHLBI; Mayo Clinic, Rochester, Minnesota

LAB/BRANCH
Laboratory of Clinical Endocrinology

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.9	.7	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We continue to study placental proteins as cancer markers. In patients with primary hyperparathyroidism, determination of the subunits of human chorionic gonadotropin may help make the diagnosis of parathyroid carcinoma or screen for associated malignant pancreatic islet disease. The subunits of CG appear to be nonspecific but particularly sensitive markers for malignant transformations of gastrinomas. Immunocytochemical demonstration of placental lactogen supports the placental origin of the syncytial giant cells in so-called "syncytial endometritis".

Placental cells transformed by temperature-sensitive mutants of SV40 virus produce low levels of pregnancy-specific beta$_1$-glycoprotein (SP$_1$) at the permissive temperature (33C), but at the restrictive temperature (40C) where the transformed phenotype was lost, SP$_1$ production was greatly enhanced. SP$_1$ exists in solution in the form of aggregates (at least as high as the trimer) that dissociate only slowly on dilution. Purified CG-α of urinary origin is heterogeneous and contains forms with terminal N-acetylglucosamine.

Project Description:

A. In Vivo Studies

The concentration of subunits of human chorionic gonadotropin (CG-α and CG-β) was determined in plasma or serum from 70 patients with primary hyperparathyroidism (1°HPT). Two of three patients with parathyroid carcinoma showed elevation in plasma concentration of both subunits which fell following surgical removal of tumor. An extract prepared from the tumor of one of these patients contained the subunits in high concentration, whereas in extracts similarly prepared from tissue removed from seven patients with benign 1°HPT, the subunits were not detectable or were present in much lower concentration. In 42 cases of benign 1°HPT, samples from veins containing a ten-fold gradient of parathyroid hormone (PTH) obtained during selective venous catheterization and peripheral samples from the same patients were analyzed for CG subunits. Only one patient demonstrated a mild elevation of CG-α in parathyroid venous effluent alone that may have represented subunit release by apparently benign parathyroid tissue. Thirty patients with multiple endocrine neoplasia type I (MEN I) were tested; eight evidenced clinically active islet cell tumors and six of these eight showed high circulating concentrations of CG-α (and CG-β in one case). Patients in the MEN I group with 1°HPT or pituitary tumors, but no evident pancreatic islet disease, did not show elevations in subunit concentrations. Thus, in patients with 1°HPT, determination of subunits of CG may be helpful in making the diagnosis of parathyroid carcinoma or in screening for associated (and probably malignant) pancreatic islet disease.

Radioimmunoassays of CG, CG-α, and CG-β in sera from 32 patients with Zollinger-Ellison syndrome (ZES) revealed elevated concentrations (above the 99th percentile of a large control group) of CG-α in 15 and CG-β in 4 cases. Neither subunit was elevated initially in 17 of 24 "benign" ZES patients, although 2 of them developed elevations later in the course of their disease. Only patients with proven hepatic or distant metastases were classified "malignant": 7 out of 8 of these had elevated CG-α, and 4 had CG-β elevations in serum. In 7 patients classified "benign", initial CG-α concentrations were elevated and remained so or rose further during 2-4 years of follow-up: two of these now have metastases and the others have evidence of locally invasive disease or an expanding mass in the head of the pancreas, without metastases. Patients with elevated CG-β appeared to have a particulary bad prognosis. Sera from 17 patients with non-malignant gastrointestinal diseases showed no elevations of either CG-α or CG-β.

The subunits of CG appear to be non-specific but sensitive markers for malignant transformations of gastrinomas and may prove valuable in the management and prognosis of ZES.

We are collecting sera from three groups of patients with increased circulating levels of immunoglobulin (multiple myeloma, Waldenstrom's macroglobulinemia and "benign" monoclonal gammopathy). These are being studied for CG, CG-α, CG-β, placental lactogen (PL), and pregnancy-specific β_1-glycoprotein (SP_1) to see whether any of these proteins will be useful as tumor markers and whether they can help discriminate between "benign" and malignant gammopathy.

We have found "pregnancy-specific beta$_1$-glycoprotein" (SP_1) in culture media of virtually all human fibroblast cel lines examined. It was not useful as a tumor marker in cases of fibro- or other sarcomas but elevated concentrations (133 ng/ml) were detected in the serum of one young man (normal: < 3.2, 95% C.L.) with metastatic fibrosarcoma. Examination of serial samples, however, revealed no correlation of serum SP_1 with body burden of tumor.

B. In Vitro Studies

One hundred endometrial biopsies of various histological patterns, and material from 10 tubal pregnancies together with their associated uterine decidua, were examined for the presence of human placental lactogen using affinity-purified first and second antibodies, and an indirect immunoperoxidase technique. Positive cells in endometrial curettings were seen only in association with an intrauterine pregnancy, and morphologically resembled syncytiotrophoblast. Decidua associated with tubal pregnancy, pseudodecidua in progestogen-treated patients, and proliferative, secretory, and basal endometria were all negative. An immunoperoxidase stain for human placental lactogen is a useful marker for intrauterine pregnancy and supports the placental origin of the syncytial giant cells in so-called "synctial endometritis". The technique is of potential value in those endometrial biopsies where pregnancy is suspected but no villi are seen.

The synthesis of SP_1 was studied in simian virus 40 (SV40) temperature sensitive A (tsA) mutant-transformed human first trimester and term placental cells. At the permissive temperature (33 C, transformed phenotype), they produced low levels of SP_1. At the restrictive temperature (40 C), the transformed phenotype was lost and the production of SP_1 was greatly enhanced. The SP_1 produced by these transformed placental cells was indistinguishable from purified placental SP_1 by its behavior on concanavalin A-Sepharose, by cochromatography on a Bio-Gel A-0.5m column, and in the slope of its RIA dose-response curve. The synthesis of SP_1 both at 33 and 40 C in these placental cells was greatly induced by sodium butyrate and 5-bromo-2'-deoxyuridine (BrdUrd). Sodium butyrate was a more effective inducer at 33 C whereas BrdUrd appeared to be a better inducer at 40 C.

The molecular properties of pregnancy-specific β_1-glycoprotein (SP_1) have been evaluated by sedimentation equilibrium, gel electrophoresis, and circular dichroic measurements. The intensity of the circular dichronic spectrum of SP_1 in the far ultraviolet is quite low with a positive maximum at 235 nm and a negative maximum at 215 nm. The 235 nm band is lost upon treatment with mixed exoglycosidases, reducing agents and guanidinium chloride, but not by treatment with neuraminidase. Treatment of SP_1 with neuraminidase or mixed exoglycosidases results in decreases in the apparent molecular weight obtained by sodium dodecyl sulfate gel electrophoresis. The molecular weight of SP_1 in 6 M guanidinium chloride (± reducing agents) by equilibrium sedimentation ws 43,600. In the absence of denaturing agents, SP_1 exists in the form of aggregates (at least as high as trimeric SP_1) that dissociate only slowly upon dilution. The presence of these aggregates may contribute to the reported molecular heterogeneity of SP_1 as found by immunoelectrophoresis.

Structural analysis of the carbohydrate moieties in alpha subunit prepared from the CG of pregnancy urine has been carried out on oligosaccharides obtained after trifluoroacetolysis. The monosaccharides were analyzed by gas-liquid chromatography-mass spectrometry after reduction and acetylation. The results of these studies are compatible with heterogeneity of urinary CG-α. In addition to the accepted structure with terminal sialic acid-α2,3-galactose, forms have been found with terminal N-acetylglucosamine on one or both antennae. Quantitation of the various forms is proceeding.

The large form of PL eluting at the void volume of dextran (Sephadex G100) and polyacrylamide (Bio Gel P 150) colums is unstable. When stored at 4°C for 3 weeks in azide, aprotinin, and phenylmethylsulfonyl fluoride, this form lost 85% of its immunoactivity. When this form was rechromatographed on G100 (after storing at 4°C in phosphate buffer with ovalbumin for 6 days), 50% of the activity had shifted to a smaller form. The chemical basis for this instability is being examined.

Publications:

1. Blackman, M. R., Weintraub, B. D., Rosen, S. W., Kourides, I. A., Steinwascher, K., and Gail, M. H.: Human placental and pituitary glycoprotein hormones and their subunits as tumor markers: A quantitative assessment. J. Natl. Cancer Inst. 65: 81-93, 1980.

2. Rosen, S. W., Kaminska, J., Calvert, I. S., Ellmore, N., and Aaronson, S. A.: Ectopic production of "pregnancy-specific" beta-1 glycoprotein in vitro: Discordance with three other placental proteins. Am. J. Obstet. Gynecol. 137: 525-529, 1980.

3. Rosen, S. W., Calvert, I., Weintraub, B. D., Tseng, J. S., and Rabson, A. S.: Stimulation by $N^6,O^{2'}$-dibutyryl cyclic adenosine 3':5'-monophosphate of ectopic production of the free β subunit of chorionic gonadotropin by a human brain tumor cell line. Cancer Res. 40: 4325-4328, 1980.

4. Rosen, S. W. and Weintraub, B. D.: Tumor Production of Gonadotropins and Their Subunits. In Cumming, I. A., Funder, J. W., and Mendelsohn, F. A. O. (Eds.): Endocrinology 1980 (Proc. VI Int. Cong. Endocrinology, Melbourne, Australia, Feb., 1980). Canberra, Australian Academy of Science, 1980, pp. 653-656.

5. Hamilton, T. A., Tin, A. W., Sussman, H. H., Weintraub, B. D., and Rosen, S. W.: Independent expression of alpha-hCG and placental alkaline phosphatase in clonal human cell lines. Oncodevelopmental Biology and Medicine 1: 161-167, 1980.

6. Heyderman, E., Gibbons, A. R., and Rosen, S. W.: Immunoperoxidase localisation of human placental lactogen: A marker for the placental origin of the giant cells in "syncytial endometritis" of pregnancy. J. Clin. Pathol. 34: 303-307, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45013-09 CEB

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biosynthesis and Glycosylation of Thyrotropin (formerly Biosynthesis of Glycoprotein Hormones)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. D. Weintraub	Medical Director	CEB NIADDK
Other:	B. S. Stannard	Biologist	CEB NIADDK
	J. Magner	Clinical Associate	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.7	PROFESSIONAL: 1.0	OTHER: 1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inhibition of TSH subunit glycosylation by tunicamycin leads to rapid intracellular proteolysis of nonglycosylated subunits. Neither nonglycosylated nor core-glycosylated α combines with normal TSH-β in cell-free combination experiments. Subcellular fractionation studies show a translational block of nascent chains at 11,000 daltons, presumably caused by glycosylation. Subunit processing occurs in both the rough and smooth endoplasmic reticulum while combination occurs in the smooth endoplasmic reticulum or golgi.

PHS-6040
(Rev. 10-76)

Project Description:

I. Studies in Dispersed Thyrotroph Cells

We have continued studies of de novo TSH biosynthesis in primary cultures of mouse thyrotropic tumor cells. In the presence of tunicamycin, which inhibits glycosylation, there is the appearance of carbohydrate-free 11 K α and 13 K β subunits. Pulse-chase studies reveal that 50% of these non-glycosylated subunits are degraded intracellularly before secretion. In contrast, neither glycosylated subunits nor other proteins present in the thyrotroph are degraded with or without tunicamycin.

In cell-free combination studies, neither the nonglycosylated 11 K α nor the core glycosylated 18 K α will combine with normal bovine TSH-β to form TSH. However, 21-23 K "mature" α shows 30-40% combining activity. Certain of these forms may have additional terminal carbohydrate residues that inhibit combination.

Currently we are attempting to characterize the carbohydrate structure of various subunit forms by the use of endoglycosidase H, an enzyme that cleaves only high mannose-containing residues. Preliminary studies indicate that 18 K α is endo H sensitive, but must be fully denatured before this enzyme can cleave the high mannose oligosaccharide.

II. Studies of Subcellular Fractions

In order to learn the subcellular sites of subunit processing and combination, differential and sucrose density gradient centrifugation were employed to isolate ribosomes with attached nascent chains as well as vesicles of rough endoplasmic reticulum (e.r.), smooth e.r. or golgi.

In one series of experiments, unlabeled nascent chains were displaced from ribosomes with ^{3}H-puromycin. Techniques of immunoprecipitation and SDS gel electrophoresis disclosed a spectrum of molecular weights for such nascent chains, with a predominance of about 11 K, the weight of unglycosylated core subunit. The pattern was suggestive of the "translational block" phenomenon seen by others in studies of immunoglobulin biosynthesis; i.e., there may be either an initiation block or pause in chain elongation at the time of dolichol addition, which favors the accumulation of nascent chains of particular molecular weights.

In other experiments, mouse thyrotropic tumor slices were incubated with ^{35}S-methionine for 10 or 60 minutes, homogenized and fractionated by centrifugation. Electron microscopy of microsomal fractions at interfaces 1.35/2.0 M, 0.86/1.35 M and 0.6/0.86 M sucrose revealed ratios of smooth or golgi vesicles: rough of $< 10:> 90$, 80:20 and $> 90:< 10$, respectively. Detergent-solubilized radioactivity was immunoprecipitated with anti-bLHα or anti-b TSHβ sera, and subunits were analyzed by SDS gel electrophoresis. Acid dissociation methods demonstrated that ^{35}S in the 21 K gel region of anti-β represented α subunit derived from intact TSH.

We found that most of the time-dependent processing of 11 K and 18 K to 21 K α occurs in the rough endoplasmic reticulum, while most of the combination of α and β subunits occurs in the smoorh e.r. or golgi. These results are consistent with previous data showing that carboydrate processing appears to be necessary for subunit combination.

Publications:

1. Weintraub, B. D.: Van Meter-Armour Prize Lecture of the American Thyroid Association: Biosynthesis, Secretion and Receptor Interaction of Thyrotropin and its Subunits. In Stockigt, J. R. and Nogataki, S. (Eds.): Thyroid Research VIII. Canberra, Australian Academy of Science, 1980, pp. 43-46.

2. Weintraub, B. D., Kourides, I. A., Stannard, B. S., Giudice, L. C., and Bridgen, P. J.: Biosynthesis of Thyrotropin: Cell-free and Intact Cell Studies. In Cumming, I. A., Funder, J. W., and Mendelsohn, F. A. O. (Eds.): Endocrinology, 1980, Proceedings of the VI International Endocrinology Congress. Canberra, Australian Academy of Science, 1980, pp. 512-515.

3. Weintraub, B. D., Stannard, B. S., Linnekin, D., and Marshall, M.: Relationship of glycosylation to de novo thyroid-stimulating hormone biosynthesis and secretion by mouse pituitary tumor cells. J. Biol. Chem. 255: 5715-5723, 1980.

4. Weintraub, B. D.: Biosynthesis and Secretion of the Alpha and Beta Subunits of TSH. In Albertine, E. (Ed.): Radioimmunoassay of Hormones, Proteins and Enzymes. Amsterdam, Excerpta Medica, 1980, pp. 23-28.

5. Marshall, M. C., Williams, D., and Weintraub, B. D.: Regulation of de novo biosynthesis of thyrotropin in normal, hyperplastic and neoplastic thyrotrophs. Endocrinology 108: 908-915, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45014-10 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Adrenal and Thyroid Secretion (formerly adrenal Secretion)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Wolff	Medical Director	CEB NIADDK
	J. Fradkin	Clinical Associate	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of adenosine in secretion has been extended to the thyroid which shows membranes that appear to lose their receptor response when isolated. In intact thyroid fragments, cAMP can be readily demonstrated to increase after exposure to adenosine analogs. The properties of this response are characteristic of an Ra adenosine receptor.

PHS-6040
(Rev. 10-76)

Project Description:

Adenosine analogs stimulate cAMP accumulation in fragments of guinea pig thyroid exposed to the nucleoside *in vitro*. Only partial agonism is observed but it follows the potency sequence N-ethylcarboxamido adenosine > 2-chloroadenosine > N^6phenylisopropyladenosine, and is antagonized by theophylline. Hence, guinea pig thyroid exhibits the properties of an Ra type of adenosine receptor. Why it remains difficult to evoke such responses in isolated membranes is under investigation.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45015-11 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Thyroid Plasma Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Wolff	Medical Director	CEB NIADDK
	A. Goldhammer	Staff Fellow	CEB NIADDK
	G. H. Cook	Chemist	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	.7	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Activation of thyroid adenylate cyclase by guanine nucleotides occurs through two kinetically distinct steps that may be related to two binding sites on one regulator protein or two different regulator proteins.

Project Description:

The relation of different physical states of solubilized adenylate cyclase to the G nucleotide regulator protein was investigated kinetically. Using App(NH)p as a substrate, modulation of fluoride stimulated activity by guanine nucleotides could be observed. In conjunction with fluoride (10 mM), GTP (10 μM) enhanced adenylate cyclase activity while GDP (10 μM) inhibited the same reaction relative to fluoride alone. Neither of three analogues Gpp(NH)p, GTPγS or GDPβS had any effect. These effects could not be observed when using ATP as the substrate with a nucleotide triphosphate regenerating system. Fluoride was shown to inhibit GTPase activities present in the membrane approximately four-fold, suggesting a possible mechanism for the synergistic behavior of fluoride and GTP.

The activation of thyroid adenylate cyclase by Gpp(NH)p was studied by steady state kinetics. Computer treatment of the data obtained over a wide range of substrate concentrations (0.03-30 μM) resulted in non-linear Eadie-Hofstee plots, suggesting the possible involvement of two independent guanine nucleotide binding sites or two sites exhibiting negative cooperativity. When GDPβS was used to look at inhibition kinetics again, non-linearity was observed and the system was not subject to simple competitive inhibition. This could be demonstrated by the lack of complete inhibition of Gpp(NH)p-stimulated cyclase; a resultant data reduction revealed that only one of the two postulated Gpp(NH)p binding sites was inhibited.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45016-11 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Thyroid Hormone Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Wolff	Medical Director	CEB NIADDK
	Y. Lee	Staff Fellow	CEB NIADDK
Other:	S. Berkowitz	Chemist	Celanese Corp.
	B. Rousset	Associate Professor	Univ. de Lyon,

COOPERATING UNITS (if any)

Faculté de Medecine Alexis Carrel, Lyon, France

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lactoperoxidase-induced splitting of α from β tubulin does not interfere with colchicine binding, suggesting only one of the subunits is required for binding. The chemical specificity of such binding is more stringent on tubulin but of higher affinity on antibodies.

PHS-6040
(Rev. 10-76)

Project Description:

The role of tubulin in secretion has led us to extensive studies of tubulin and its interactions with colchicine and calcium. It was found that lactoperoxidase binds to tubulin by a heme-SH interaction which leads to dissocation of the α and β subunits. We conclude that the colchicine binding activity and the polymerization capacity of tubulin are differently altered by the dissociation of the α and β subunits. Lactoperoxidase does not affect colchicine binding. Since a maximum of 1 colchicine is bound/dimer, the colchicine binding site is probably located on only one of the two tubulin subunits and the presence of the other is not obligatory. On the other hand, binding of lactoperoxidase interferes with the ability of tubulin to polymerize by a mechanism that appears to involve the dissociation of the subunits and possibly other effects as well.

The binding interaction has also permitted a rapid purification of tubulin. Brain tubulin binds to lactoperoxidase coupled to Affigel 10 through a 10 Ᾰ succinylated aminoalkyl spacer and can be eluted by an ionic strength gradient. The tubulin can be obtained about 90% electrophoretically pure in 2 to 3 h without glycerol or GTP. It retains its ability to bind colchicine. Compared to tubulin purified by the assembly disassembly procedure, affinity-purified tubulin has a higher critical concentration for polymerization and the purified protein appears to be free of high molecular weight microtubule-associated proteins. Tubulin binding to the lactoperoxidase affinity column protects the colchicine binding site against decay at 4°C, whereas the interaction of tubulin with soluble lactoperoxidase does not.

We have also compared colchicine binding to tubulin with that to specifically prepared antibodies. Antibodies to colchicine have been prepared by injecting rabbits with an antigen prepared by coupling deacetylcolchicine to bovine serum albumin with a water-soluble carbodiimide. Sera of high titer were obtained with apparent dissociation constants for colchicine ranging from 5 to 17 nM Binding was rapid and occurred at low temperatures. The chemical specificity of the binding site(s) resembled that in tubulin for the A and B rings of colchicine. Unlike tubulin, the antibody binding site tolerated numerous changes in the tropolone moiety (C ring). In contrast to tubulin, binding of colchicine to the antibody was high enough to prevent colchicine fluorescence in the presence of tubulin and to restore polymerization in colchicine-inhibited tubulin preparations.

Finally, the role of Ca^{2+} in tubulin polymerization has been investigated. We find that factors that promote nucleation such as increased tubulin concentration or temperature, addition of microtubule seeds or 16 S oligomers decrease the intrinsic high Ca^{2+} sensitivity for inhibition of polymerization. Calmodulin enhances that sensitivity. The precise contribution of microtubule-associated proteins to Ca^{2+} and calmodulin sensitivity is currently under study.

Publications:

1. Rousset, B. and Wolff, J.: Effect of lactoperoxidase-induced α-β tubulin splitting on colchicine binding and polymerization of brain tubulin. FEBS Letters 115: 235-238, 1980.

2. Rousset, B. and Wolff, J.: Purification of brain tubulin by affinity chromatography on immobilized lactoperoxidase. J. Biol. Chem. 255: 11677-11681, 1980.

3. Wolff, J., Capraro, H.-G., Brossi, A., and Cook, G. H.: Colchicine binding to antibodies. J. Biol. Chem. 255: 7144-7148, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45018-06 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Adenylate Cyclase and Other Extracellular Products of B. Pertussis: Effects on Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Wolff	Medical Director	CEB NIADDK
	A. Goldhammer	Staff Fellow	CEB NIADDK
	H. G. Cook	Chemist	CEB NIADDK
	M.-C. Kilhoffer	Visiting Fellow	CEB NIADDK
Other:	E. Hewlett	Asst. Prof. of Med.	U. of Virginia, Charlottesville
	C. R. Manclark	Research Microbiologist	FDA

COOPERATING UNITS (if any)

Department of Medicine, University of Virginia Medical School; Division of Bacterial Products, Bureau of Biologics, FDA

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.9	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adenylate cyclase of *B. pertussis* culture media is stimualted 3-10 fold by various amphiphiles. Stimulation is not critically dependent on polar bead groups, fatty acid chain length or critical micelle concentration. The sensitivity toward calmodulin is enhanced by amphiphiles.

Project Description:

Bordetella pertussis organisms secrete a soluble adenylate cyclase into the periplasmic space whence the enzyme finds its way into the culture medium. The activity associated with the organisms or the culture medium is markedly influenced by growth conditions of the organism due to the presence of an activator in some of the media and not others. This activator has been identified as calmodulin. Activatability of the enzyme by Ca^{2+}/calmodulin appears to be stable in the intact, stored organisms and in crude culture supernatants. The latter enzyme can be purified but concomitant with purification it progressively loses the ability to become stimulated by Ca^{2+}/calmodulin. We, therefore, undertook a search for an additional factor required to promote or permit the activation by calmodulin.

In the absence of calmodulin, the adenylate cyclase activity of Bordetella pertussis culture supernatants is activated 3-10 fold by various amphiphiles including many classes of phospholipids and nonionic detergents. Gangliosides are inhibitory. The stimulation affects the V_{max} and not the K_m. Neither the nature of the polar head group, the length of the fatty acid chains, nor the hydrophile-lipophile balance (in the Triton X series) are major determinants for activation. Short-chain lecithins activate as monomers, whereas long-chain lecithins activate only above the critical micelle concentrations, suggesting high affinity hydrophobic binding sites.

Judged by EGTA inhibition, the amphiphile-mediated activation requires Ca^{2+} in the absence of calmodulin. In addition, amphiphiles sensitize the adenylate cyclase to Ca^{2+}/calmodulin and are also synergistic with calmodulin for maximal stimulation.

We have also found that a variety of proteins and tissue preparations (rabbit erythrocyte lysate, catalase, peroxidase, creatine phosphokinase, and lima bean trypsin inhibitor) contain protein activator(s) of the extracellular adenylate cyclase of intact Bordetella pertussis organisms. Stimulation of adenylate cyclase activity of up to 1000-fold over basal activity can be obtained. Activation of the adenylate cyclase is due to the presence of calmodulin in these protein preparations. The criteria to establish this were: Ca^{2+} dependence of the activation, inhibition by trifluoperazine, heat stability of the activator, chromatographic behavior like authentic calmodulin, and stimulation of cyclic nucleotide phosphodiesterase by the activators. The great sensitivity of the B. pertussis adenylate cyclase assay makes this an ideal system for the detection of trace amounts of calmodulin, in the presence of large amounts of other proteins.

Publications:

1. Wolff, J., Cook, H. G., Goldhammer, A. R., and Berkowitz, S. A.: Calmodulin activates prokaryotic adenylate cyclase. Proc. Natl. Acad. Sci. USA 77: 3841-3844, 1980.

2. Goldhammer, A. R., Wolff, J., Cook, G. H., Berkowitz, S. A., Klee, C. B., Manclark, C. R., and Hewlett, E. L.: Spurious protein activators of *Bordetella pertussis* adenylate cyclase. Eur. J. Biochem. 115: 605-609, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45021-05 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Pathophysiology of Pituitary Gonadotropins (formerly Pituitary Gonadotropic Pathophysiology)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. W. Rosen	Medical Director	CEB NIADDK
Other:	J. M. Lieblich	Director, Med. Educ.	Highland Park Hosp., IL
	A. S. Rogol	Assoc. Prof. Ped.	Univ. Va. Sch. of Med., VA
	B. White	Sr. Investigator	LEP NIADDK
	J. Morrow	Clinical Associate	CEB NIADDK

COOPERATING UNITS (if any)

Highland Park Hospital, Department of Medicine, IL; University of Virginia School of Medicine, VA

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .5	PROFESSIONAL: .4	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies continue on syndromes of hypogonadotropic hypogonadism with anosmia (the Kallmann syndrome) and without anosmia, as well as on patients with anosmia who are clinically eugonadal. Episodic secretion of pituitary gonadotropins is being monitored in selected patients from all three groups.

PHS-6040
(Rev. 10-76)

A. The Syndrome of Hypogonadotropic Hypogonadism and Anosmia (HHA): The Kallman Syndrome

Two additional cases of HHA have been recognized in the large kindred from Vermont that we have been studying for several years. One of these siblings was investigated here; his sister has been studied elsewhere. Of particular interest was the documentation of hypogonadotropic hypogonadism (HH) in his paternal aunt (who has a normal sense of smell); another distant relative with HH and normal olfaction has also been identified in this kindred. Moreover, examination of old church records has disclosed that this new sibship was consanguineous, linked by a marriage six generations earlier.

We are also studying the episodic secretion of pituitary gonadotropins by patients with HHA. Usually, the hypogonadotropinemia of patients with HHA is due to a decrease in both amplitude and frequency of secretory pulses. However, there appears to be some heterogeneity - much as there is clinical heterogeneity in these syndromes - and there may be subsets of patients with differences in secretion of LH versus FSH, or with decreased amplitude but normal frequency, etc. As controls, we are studying both patients with HH and with isolated congenital anosmia.

Publications:

1. Rogol, A. D., Mittal, K. K., White, B. J., McGinniss, M. H., Lieblich, J. M., and Rosen, S. W.: HLA-compatible paternity in two "fertile eunuchs" with congenital hypogonadotropic hypogonadism and anosmia (the Kallman syndrome). J. Clin. Endocrinol. Metab. 51: 275-279, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45023-05 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Thyrotropin Receptor-Binding and Action: Relationship to Anti-thyroid Immunoglobulins (formerly TSH Binding Sites: Relationship to Anti-thyroid Immunoglobulins)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. D. Weintraub	Medical Director	CEB NIADDK
Other:	L. Joshi	Visiting Fellow	CEB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Heterogeneous forms of bovine, mouse and human TSH show differences in receptor binding and cyclase activating properties. Certain of these forms may be competitive inhibitors of the action of TSH. Lymphapheresis and plasmapheresis appear to be of moderate benefit in the treatment of Graves' ophthalmopathy.

PHS-6040
(Rev. 10-76)

Project Description:

I. Heterogeneous Forms of Mouse and Bovine TSH

We have continued to study the receptor-binding as well as the adenylate cyclase activation of heterogeneous forms of TSH derived from various sources. Mouse thyrotropic tumor, sera and media as well as partially and highly purified preparations of bovine pituitary TSH have been fractionated on long columns of Sephadex G 100. Each of these preparations show multiple forms which differ in their receptor binding and adenylate cyclase stimulating properties. Certain of these forms with high receptor binding/cyclase ratios appear to act as competitive inhibitors to the cyclase stimulation caused by standard bovine TSH. We believe that these heterogeneous forms differ in carbohydrate and that proper glycosylation is necessary for coupling the TSH receptor to adenylate cyclase.

II. Human TSH with Impaired Biologic Activity

We have found various forms of human serum TSH with normal receptor binding but poor activation of cyclase. One such form is a high molecular weight TSH which appears to be bound to a protein component of serum. Another such form is present in sera from patients with hypothalamic TSH deficiency. This form of TSH is of normal molecular weight but may be deficient in terminal carbohydrate residues.

III. Effect of Cytopheresis in Graves' Disease

We have now treated 7 patients with Graves' exophthalmos with a six-week course (two procedures per week) of either plasma- or lymphapharesis. Certain of the patients have had moderate subjective and objective improvement in ophthalmopathy during the procedures. Two of the patients have shown complete remission of their thyroid-binding immunoglobulins. We are currently studying the effects of these procedures on thyroid as well as immune function.

Publications:

1. Pekonen, F., Williams, D. M., and Weintraub, B. D.: Purification of thyrotropin and other glycoprotein hormones by immunoaffinity chromatography. Endocrinology 106: 1327-1332, 1980.

2. Kourides, I. A., Pekonen, F., and Weintraub, B. D.: Absence of thyroid-binding immunoglobulins in patients with thyrotropin-mediated hyperthyroidism. J. Clin. Endocrinol. Metab. 51: 271-274, 1980.

3. Pekonen, F. and Weintraub, B. D.: Salt-induced exposure of high affinity thyrotropin receptors on human and porcine thyroid membranes. J. Biol. Chem. 255: 8121-8127, 1980.

4. Spitz, I. M., LeRoith, D., Hirsch, H., Carayon, P., Pekonen, F., Liel, Y., Sobel, R., Chorer, Z., and Weintraub, B. D.: Increased high molecular weight thyrotropin with impaired biologic activity in a euthyroid man. New Engl. J. Med. 304: 278-282, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45024-05 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Metabolism of Thyroid Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
	H. J. Cahnmann	Scientist Emeritus	CEB NIADDK
Other:	M. Phyillaier	Biologist	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.4	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Primary cultured rat hepatocytes were found to resemble normal hepatocytes in their metabolism of thyroid hormones by deiodination and conjugation. These cells were used to investigate the regulation of thyroxine deiodination. Insulin was found to stimulate 5'-deiodination. Glucagon prevented the insulin effect, but did not alter deiodination in the absence of insulin. Glucose had no effect and did not alter the effect of the hormones. These findings may explain the inhibition of T_4 conversion to 3,5,3'-triiodothyronine observed during fasting, and its restoration by refeeding, in the whole animal.

A new synthesis for inner-ring labeled triiodothyronine has been devised.

Project Description:

The mammalian organism has an apparent adaptation mechanism for thyroid hormone metabolism by which the conversion of thyroxine (T_4) to biologically active 3,5,3'-triiodothyronine (T_3) is decreased in fasting and increased by refeeding, especially of carbohydrate. We investigated the mechanism of this effect in primary monolayer cultures of adult rat hepatocytes. These cultured rat hepatocytes showed deiodination patterns more closely resembling those in normal liver compared to the monkey hepatocarcinoma cells used in our earlier studies. These cells also carried out conjugation with glucuronide as well as with sulfate, in contrast to the tumor cells.

Incubation of the rat hepatocytes with combinations of insulin, glucagon and/or glucose for 5 hours showed that insulin stimulated T_4 5'-deiodination, whereas glucagon inhibited the insulin stimulation but had no effect in the absence of insulin. Glucose had no effect and did not alter the effect of the hormones. This insulin effect had not been detected previously because it requires several hours in order to develop. These data suggest that the inhibition of T_4 5'-deiodination observed during fasting, and its restoration by refeeding, may be related to insulin and glucagon release rather than being dependent on glucose *per se*.

Inner-ring labeled 3,5,3'-triiodothyronine is a useful tracer for deiodination studies, but has been difficult to obtain. A method has been devised in which this compound is synthesized by the standard coupling reaction to make inner-ring labeled thyroxine, followed by controlled partial deiodination of the phenolic ring.

Publications:

1. Sato, K. and Robbins, J.: Thyroid hormone metabolism in cultured monkey hepatocarcinoma. J. Biol. Chem. 255: 7347-7352, 1980.

2. Sekura, R. D., Sato, K., Cahnmann, H. J., Robbins, J., and Jakoby, W. B.: Sulfate transfer to thyroid hormones and their analogs by hepatic aryl sulfotransferases. Endocrinology 108: 454-456, 1981.

3. Sato, K. and Cahnmann, H. J.: Synthesis of [3,5-^{125}I]triiodo-L-thyronine of high specific activity. Anal. Biochem. 102: 237-242, 1980.

4. Pangaro, L., Burman, K. D., Wartofsky, L., Cahnmann, H. J., Smallridge, R. C., O'Brian, J. T., Wright, F. D., and Latham, K.: Radioimmunoassay for 3,5-diiodothyronine and evidence for dependence on conversion from 3,5,3'-triiodothyronine. J. Clin. Endocrinol. Metab. 50: 1075-1081, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45025-03 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effects of Triiodothyronine on Liver Nucleoproteins (formerly The Effects of Triiodothyronine on Liver Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Rall	Director, Intramural Research Programs	ODIRP NIADDK
	V. M. Nikodem	Staff Fellow	CEB NIADDK
Other:	B. L. Trus	Research Chemist	CR CLS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.0	OTHER: .7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two-dimensional gel electrophoresis of the changes in nucleoprotein of rat liver was extended through the development of computerized quantitation of the different proteins. Of some 500 proteins, 102 either disappeared or were markedly reduced in livers from hypothyroid rats. These changes are the greatest in number and in quantity ever reported as an effect of thyroid hormone. Additional studies on post-transcriptional modification of nucleoproteins showed that thyroidectomy inhibited acetylation, phosphorylation and ADP ribosylation of many of these proteins. Since some of these reactions may affect gene activity and since, in general, actively transcribed genes are more sensitive to DNase I and micrococcal nuclease, we investigated the effect of thyroid states on chromatin sensitivity to nucleases. We found that hypothyroidism was associated with decreased solubilization of liver chromatin to both DNase I and micrococcal nuclease.

PHS-6040
(Rev. 2-81)

1. We have extended our studies on the labeling of the thyroid hormone receptor from rat liver nuclei to obtain additional proof of the specificity of N-bromoacetyl (BrAc) derivatives of L-thyroxine (T_4) and 3,3'5-triiodo-L-thyronine (T_3).

   It is clear from binding studied by Sephadex gel chromatography and HPLC that BrAc derivatives of T_3 or T_4 compete for the same binding site in the nuclear receptor from rat liver. The same chromatographic pattern after HPLC and identical electrophoretic mobilities in nondenaturing gels of [^{125}I]T_3-bound and Ac[^{125}I]T_3-labeled nuclear receptor showed that BrAc[^{125}I]T_3 labeled the nuclear receptor. Furthermore, the inability of BrAc[^{125}I]T_3 to label heat-treated nuclear receptor is additional proof of the specificity in labeling of the T_3 binding sites. Gel electrophoresis of Ac[^{125}I]T_3- or Ac[^{125}I]T_4-labeled nuclear receptor in the presence of denaturing agents under reducing conditions indicated that a single protein species was covalently labeled with the BrAc derivatives. We found that Ac[^{125}I]T_3- and Ac[^{125}I]T_4-labeled nuclear receptor protein showed the same chromatographic pattern and identical electrophoretic mobilities and thus demonstrated that T_3 and T_4 bind to the same protein, which has a molecular weight of 56,000.

2. Quantitative changes in nuclear proteins from control, thyroidectomized and T_3-treated thyroidectomized rats was accomplished by means of computer analysis of two-dimensional gels.

   A computer program was designed to measure the relative concentration of each protein. Approximately 500 protein subunits could be distinguished. Of these, 102 either disappeared or were markedly decreased after thyroidectomy. Shortly after administration of T_3 (6 hours), 13 proteins reappeared and by 24 hours, 67 additional proteins had reappeared. These changes in protein concentrations are both the greatest in number and the largest reported as an effect of thyroid hormone. Twenty-four hours after T_3 administration, some 22 proteins seen in normal liver could not be found. Two new proteins appeared in thyroidectomized rat liver nuclei. We conclude that a major and rapid effect of thyroid hormone is exerted upon hepatic nucleoproteins.

3. The effect of thyroid hormone on acetylation, phosphorylation and ADP-ribosylation of rat liver nucleoproteins was studied by incubating intact nuclei with labeled precursors. Acetylation, which occurred in histones and low molecular weight proteins (< 20,000), was depressed in nuclei from thyroidectomized animals. The administration of T_3 increased acetate incorporation to 50% over contorl levels. Incorporation of labeled phosphate from ATP in control animals was found in nucleoproteins ranging in molecular weight from that of histones to greater than 100,000. Incorporation into most proteins was decreased in nuclei from thyroidectomized animals and increased by the administration of T_3. The greatest increase produced by T_3 (to 140% of

control values) was seen in proteins of molecular weight greater than 68,000. Incorporation of labeled ADP ribose from NAD was seen in many different nucleoproteins and was particularly prominent in a protein of 103,000. Nuclei from thyroidectomized animals incorporated less ADP ribose in most proteins. Both high molecular weight proteins (> 68,000) and low molecular weight proteins (< 30,000) showed a further decrease in ADP ribose incorporation in nuclei from thyroidectomized rats given T_3. However, a few proteins of the middle molecular weight class showed increased ADP incorporation subsequent to the injection of T_3. We suggest that a generalized increase in protein synthetic rates previously noted to be caused by T_3 may be due to increased acetylation and phosphorylation of histones and other proteins which accelerate transcription of already active genes.

4. The above studies showed that some proteins were more phosphorylated or acetylated than others after T_3 treatment. Using these results and knowing that (1) transcriptionally active regions of DNA show a higher degree of acetylation and phosphorylation and that (2) micrococcal nuclease and deoxyribonuclease I preferentially digest transcriptionally active genes, we investigated differences in the rate of digestion of DNA from rat liver nuclei of control, thyroidectomized and T_3-treated thryoidectomized rats. Freshly prepared nuclei were adjusted to 20 OD_{260} in .5 ml of incubation buffer containing Ca^{++} and Mg^{++} and incubated with micrococcal nuclease (400 U) or deoxyribonuclease I (30 U) on ice for different times. Digestion was stopped with EDTA and the amount of DNA released in the supernatant was determined by fluorometric assay. The preliminary results indicate that, 24 h after injection of T_3 (1 mg/100 g body weight) to thyroidectomized rats, the rate of digestion by micrococcal nuclease reached 140% when compared with nuclei prepared from thyroidectomized rats. Digestion with deoxyribonuclease I followed basically the same pattern. At the present time, we are studying a T_3 dose and time response.

Publications:

1. Nikodem, V. M., Cheng, S.-Y., and Rall, J. E.: Affinity labeling of rat liver thyroid hormone nuclear receptor. Proc. Natl. Acad. Sci. USA 77: 7064-7068, 1980.

2. Robbins, J., Rall, J. E., and Gorden, P.: The Thyroid and Iodine Metabolism. In Bondy, P. K. and Rosenberg, L. E. (Eds.): Metabolic Control and Disease. Philadelphia, W. B. Saunders, Company, 1980, pp. 1325-1425.

3. Nikodem, V. M., Trus, B. L., and Rall, J. E.: Two-dimensional gel analysis of rat liver nuclear proteins after thyroidecotmy and thyroid hormone treatment. Proc. Natl. Acad. Sci. USA, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45027-03 CEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Synthesis of Thyroid Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. J. Cahnmann	Scientist Emeritus	CEB NIADDK
	K. Sato	Visiting Associate	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Biochemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .0	PROFESSIONAL: .0	OTHER: .0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Terminated

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45028-03 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Thyroid Hormones-Cell Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Robbins	Chief, Clin. Endo. Br.	CEB NIADDK
	S.-Y. Cheng	Research Chemist	LMB NCI
Other:	E. Movius	Medical Staff Fellow	CEB NIADDK
	M. Phyillaier	Biologist	CEB NIADDK

COOPERATING UNITS (if any)
Laboratory of Molecular Biology, National Cancer Institute

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.9	.4	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rhodamine-labeled triiodothyronine and isopropyl-diiodothyronine were shown to enter monkey hepatocarcinoma cells growing as monolayer cultures, and to accumulate in the nucleus in a manner similar to the parent hormone. This system should be useful for further study of the mechanism by which thyroid hormones enter the mammalian hepatocyte and other cells.

PHS-6040
(Rev. 2-81)

Project Description:

In previous work, a fluorescent derivative - rhodamine-labeled triiodothyronine - was used to study cell uptake of thyroid hormone. Using video-intensification fluorescence microscopy, receptor-mediated uptake in coated pits, and subsequent endocytosis, was demonstrated in cultured fibroblasts. Nuclear localization, however, could not be identified. The present study examined the interaction of rhodamine-T_3 and related compounds with monolayer cultures of monkey hepatocarcinoma cells. ^{125}I-labeled T_3, rhodamine-T_3 and rhodamine isopropyl-T_2 were used, and the cell nuclei were isolated and counted. It was shown that all compounds were incorporated into the nucleus, reaching equilibrium in 2 hours. Thus, the fluorescent derivatives reach the nuclear receptor in a manner similar to that shown by the parent hormone. Their affinity for the receptor, however, is 2 orders of magnitude lower. This system should be useful for further study by fluorescence microscopy.

Publications:

1. Cheng, S.-Y., Maxfield, F. R., Robbins, J., Willingham, M. C., and Pastan, I.: Receptor mediated uptake of 3,5,3'-triiodothyronine by cultured fibroblasts. Proc. Natl. Acad. Sci. (USA) 77: 3425-3429, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45029-02 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

POEMS Syndrome (formerly Hyperpigmentation Gammopathy Syndrome)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. W. Rosen	Medical Director	CEB NIADDK
Other:	J. Morrow	Clinical Associate	CEB NIADDK
	D. Huston	Clinical Associate	ARB NIADDK
	E. Schaefer	Sr. Investigator	MDB NHLBI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .5	PROFESSIONAL: .4	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We continued to investigate the mechanisms of hyperpigmentation and of hyperprolactinemia in a woman with the POEMS syndrome.

PHS-6040
(Rev. 2-81)

Project Description:

We continue to study M.K., a 48-year-old white woman of Italian ancestry who has a syndrome characterized by hyperpigmentation and thickening of the skin, hyperprolactinemia and galactorrhea, splenomegaly, and a circulating monoclonal IgG kappa light chain. Recently, Bardwick et al. (Medicine 59: 311, 1980) reported on 39 patients from the literature and 2 patients of their own who had a similar syndrome. They identified the common features in these patients as polyneuropathy, organomegaly, endocrinopathy, M-component, and skin changes and they named this the POEMS syndrome. Two of their patients, like our own, had unexplained hyperprolactinemia. Many patients in the literature had oligomenorrhea/amenorrhea, or impotence, but their prolactin (PRL) status was not reported. M.K. differs from most other POEMS patients because she has no neuropathy, and because she has a kappa-type M-component (rather than the lambda-type usually seen in this syndrome).

We are attempting to answer the following questions: 1) Does the monoclonal IgG stimulate melanin production? 2) What is the etiology of the hyperprolactinemia? 3) Is hyperprolactinemia a common finding in patients with the POEMS syndrome?

Melanin production by melanoma cells in vitro was not augmented by M.K.'s serum, or by her IgG, as measured in a spectrophotometric assay (Dr. Abla Creasey). A more sensitive technique for assessing melanin synthesis is by measuring the incorporation of ^{14}C-tyrosine into melanin, and we plan to use this method with the help of Dr. J. R. Whittaker.

M.K.'s IgG could increase her serum prolactin by enhancing PRL production or decreasing its clearance. The latter is unlikely: We found that IgG^{MK} did not bind ^{125}I-PRL in vitro. Direct stimulation of pituitary lactotropes is also unlikely since IgG^{MK} did not enhance in vitro PRL production by a PRL-producing line (GH$_3$) derived from a rat pituitary tumor (Tashjian). Since dopamine is known to inhibit PRL release from cultured pituitary cells, we plan to test the hypothesis that IgG^{MK} blocks the dopamine receptor.

Impotence and amenorrhea have been found in many patients with the POEMS syndrome and are also common symptoms in people with hyperprolactinemia. We plan to determine the serum PRL in several other patients with the POEMS syndrome to see if hyperprolactinemia is more common in this syndrome than presently recognized.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45030-01 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biosynthesis and Glycosylation of Human Chorionic Gonadotropin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. D. Weintraub	Medical Director	CEB NIADDK
	S. W. Rosen	Medical Director	CEB NIADDK
Other:	J. S. Morrow	Clinical Associate	CEB NIADDK
	Y. Jones-Brown	Staff Fellow	CEB NIADDK
	H. G. Fein	Staff Physician	Walter Reed Army Med. Ctr.

COOPERATING UNITS (if any)

Walter Reed Army Medical Center, Washington, D. C.

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.9	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Messenger RNA has been isolated from HeLa cells and used to direct the cell-free translation of hCG-α subunit. Glycosylation of hCG subunits in choriocarcinoma cells has been studied by the incorporation of labeled carbohydrates combined with gel filtration of oligosaccharides obtained by pronase digestion. The combining properties of exoglycosidase-treated and ectopic hCG subunits have been studied in cell-free combination assays.

Project Description:

I. Cell-free translation of messenger RNA

We are studying the *in vitro* biosynthesis of the common α-subunit of the glycoprotein hormones. Ectopic messenger RNA has been isolated from HeLa cells and translated in a wheat germ extract system. We plan to compare, by physicochemical methods, the ectopic product of cell-free translation with eutopic products from placenta and from the choriocarcinoma cell line, JEG-3. Using cell-free translation as a tool to quantitate total messenger RNA activity, we then plan to investigate putative differences in the augmentation of ectopic and eutopic production by various agents such as sodium butyrate, cyclic AMP and GnRH.

II. Glycosylation in Choriocarcinoma Cells

We have used labeled amino acids and carbohydrates to study the eutopic biosynthesis of hCG by JEG-3 choriocarcinoma cells. Unequivocal incorporation of ^{3}H-fucose into secreted and intracellular α-subunit was shown by specific immunoprecipitation. The presence of fucose in the primary biosynthetic product of the trophoblastic cell was not predicted by the currently accepted structure of α-subunit. The latter (fucose-free) structure was based, however, on analyses of α-subunit derived from pregnancy urine, demonstrating again the physiologic relevance of the biosynthetic approach.

We are now in the process of characterizing the oligosaccharide chains found on the various primary extracellular forms of hCG subunits (intact β, intact α and free α) after pronase digestion. Physiologically it is important to identify the oligosaccharide chains found on the biosynthetic products initially released from the cells. Preliminary experiments indicate that, in the presence of carrier gamma globulin, pronase digestion completely degrades the subunits' polypeptide chain. We will conduct studies in which radioactively labeled carbohydrate precursors are incorporated into hCG. The labeled hCG subunits will be specifically isolated and analyzed to determine the molecular size and to determine the structure of the oligosaccharide chains found on free and combined subunits.

III. Combining Properties of hCG Subunits

We have continued cell-free combination studies of exoglycosidase-treated α and β subunits, which show inhibition of combining properties. Currently we are investigating the role of specific carbohydrate residues by treating subunits with individual exoglycosidase enzymes including neuraminidase, galactosidase, mannosidase, etc. We have also examined the combining properties of ectopic hCG-β subunits secreted *in vitro* by cancer cell lines of Drs. Hussa and Patillo. Certain iso-subunits (differing in negative charge) appear to combine well while others combine poorly with standard hCG-α.

Publications:

1. Fein, H. G., Rosen, S. W., and Weintraub, B. D.: Increased glycosylation of serum human chorionic gonadotropin and subunits from eutopic and ectopic sources: Comparison with placental and urinary forms. J. Clin. Endocrinol. Metab. 50: 1111-1120, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45031-01 CEB

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Photolysis of Thyroxine and Thyroxine Analogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ben van der Walt	Visiting Fellow	CEB NIADDK
	Hans J. Cahnmann	Scientist Emeritus	CEB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Photolysis of thyroxine and thyroxine analogs in the near ultraviolet leads to stepwise deiodination. The 5'-iodine atoms are most easily removed. Removal of the other iodine atoms and diphenyl ether splitting takes place much more slowly. The method can be used for the preparation of specifically labeled metabolites of T_4.

PHS-6040
(Rev. 2-81)

Project No. Z01 AM 45031-01

Project Description:

Since photolysis of 3,5,3'-triiodothyronine (T_3) and thyroxine (T_4) with short-wave ultraviolet light causes extensive degradation of these hormones, we examined the use of much less energetic long-wave ultraviolet light. T_4 and various T_4 analogs were irradiated with light above 330 nm. The irradiated products were analyzed by HPLC which completely resolved a mixture of tyrosine, thyronine, and nine iodoamino acids. Only 3,3'-diiodothyronine (3,3'-T_2) and 3',5'-diiodothyronine were not well separated from each other. A comparison of various solvents showed that photodegradation proceeds more readily in methanol than in aqueous buffers. Methanol containing 1% concentrated NH_4OH was used for most irradiations. Irradiation periods for 1-4 h at room temperature gave good yields of primary degradation (deiodination) products. Photolysis converted T_4 to T_3, tetraiodothyroacetic acid (Tetrac) to 3,5,3'-triiodothyroacetic acid, and 3,3',5'-triiodothyronine (rT_3) to 3,3'-T_2. Further degradation of the primary photolysis products proceeded only slowly. Only minimal diphenyl ether splitting was observed unless irradiation periods were extended to twenty hours or longer. The observed degradation patterns show that the iodine atoms in the phenolic ring are more susceptible to photodeiodination than those in the nonphenolic ring. Photolysis for 4 h of [3-^{125}I]rT_3 gave inner-ring labeled 3,3'-T_2 and labeled 3-T_1 in 65% and 3% yield, respectively. After 20 h of irradiation, no starting material remained and the yields of 3,3'-T_2 and 3-T_1 were 42% and 13%, respectively. Photolysis of [3,5-^{125}I]T_4 for 4 h gave inner-ring labeled T_3 in 46% yield. Thus, photolysis in the near ultraviolet can be used for the preparation of specifically labeled metabolites of T_4 which are not easily obtainable by chemical synthesis.

Publications:

None

ANNUAL REPORT 1980 - 1981
Diabetes Branch, NIADDK

Recognition of Previous Work

Members of the Diabetes Branch were recognized by several awards: The Gairdner Foundation Award for Scientific Merit; the Morrison Award in Natural Sciences of the New York Academy of Science; the William H. Creasy Visiting Professorship of Clinical Pharmacology sponsored by the Burroughs Wellcome Fund; the Eli Lilly Award of the American Diabetes Association; the Drew University Award for Scientific Research; and the Joslin Medal of the New England Diabetes Association.

In addition, grants were awarded to the Branch by the Washington D. C. Affiliate of the American Diabetes Association and from the national organization of the Juvenile Diabetes Foundation. Achievements by alumni of the Branch were also recognized including a professorship at the University of Ancona and the Minkowski Prize of the European Association for the Study of Diabetes. A member of the Branch was a Plenary Lecturer at the Laurentian Hormone Conference while another was a Visiting Professor courtesy of the Italian government to visit diabetes centers throughout that country. Furthermore, one of the Section Chiefs was recruited by Harvard University and the Peter Bent Brigham Hospital to head their diabetes teaching and research program and to head the Joslin Research Foundation at the Joslin Clinic.

Insulin and Related Hormones

Evolutionary Origins of Vertebrate Hormones: We have previously shown that fruit flies and earthworms contain material that is very similar to insulin. Thus, the pancreas is not essential for the production of insulin. To extend these studies, a ciliated protozoan, Tetrahymena pyriformis, was raised in an artificial defined medium that was free of any macromolecules or serum. Extracts of the protozoa were gel filtered and found to contain insulin immunoreactivity. The immunoreactive insulin content predicted its bioactivity in isolated fat cells of rats, and the bioactivity was largely neutralized by antibodies directed against mammalian insulin. These data suggest very strongly that the bioactivity was being exercised by molecules that have insulin immunoactivity. The bioactivity was also neutralized by anti-receptor antibodies that block the action of authentic insulin, which would indicate that the bioactivity exerted by these extracts was being exercised through the insulin receptor.

Similar materials which also closely resemble mammalian insulin were demonstrated in extracts of two other unicellular eukaryotes, Neurospora crassa and Aspergillus fumigatus, two fungi. In addition, E. Coli K-12 were grown in defined medium and were also found to contain material that closely resembled mammalian insulins.

Numerous other hormones that had been thought to have been of vertebrate origin were also detected in extracts of unicellular organisms. Material that is extremely similar to ACTH 1-39 was detected by radioimmunoassay; it was very similar to ACTH 1-39 in its reactivity with specific antibodies as well as its migration in several gel systems. The content of immunoreactive ACTH predicted the bioactivity of this material measured as stimulation of the release of corticosterone from isolated rat adrenal cells. In addition, several opiate-like materials were detected by specific radioimmunoassays or by radioreceptor assays. In addition, high molecular weight components were demonstrated that had reactivity with both ACTH and endorphin antibodies.

Somatostatin, which is both a neuropeptide and a hormone, was also detected in unicellular organisms. The material closely simulated the effect of somatostatin on rat pituitary cells and this effect was obliterated by antibodies directed against synthetic somatostatin.

Multiple other hormones have been detected by specific radioimmunoassays but the characterization is as yet less well advanced than those described above. These findings suggest that the origins of many vertebrate hormones is much earlier than previously thought, i.e. these hormonal agents did not originate in primitive vertebrates or in complex invertebrates but rather originated in the simplest unicellular organisms. The finding of these materials in simple organisms that lack nervous systems also strongly argues against a widely propounded thesis that the nervous system is the direct ancestor of the endocrine system. The present data would suggest that both of these two coordinating systems are descended from a single more primitive system.

Insulin and Insulin Receptors

Morphological Correlates of Ligand Binding to Receptors: Extensive studies of hormone binding to its receptor done by this and other laboratories typically failed to take into account changes in the cellular localization of the ligand or nature of the ligand following interaction with the target cell. Over the last several years, Gorden, et al. introduced methods to study directly the morphological correlates at the cellular level of hormone (and other ligand) binding to specific cell surface receptors. The most recent studies, which represented an extension of their own previous studies indicate that insulin binding to cultured lymphocytes occurred randomly over the whole surface of the cell, but the ligand, after binding, redistributes toward the Golgi pole of the cell. In other studies, glucagon and growth hormone binding to receptors on one or more different types of target cells were studied in detail in an attempt to characterize in greater depth the events following hormone binding to receptors. In particular, the problems of non-dissociability, its physiological meaning and possible relationship to down-regulation, were studied in detail.

Comparison of the Growth Promoting and Metabolic Effects of Insulin: Previous studies had shown that the potencies of insulins from different species as growth promoting agents were more highly conserved than were their potencies as metabolic agents. This suggests that the affinity of the insulin molecule for growth type receptors is more highly constrained than the affinity for the metabolic type of receptors.

In an extension of these studies guinea pig insulin stimulated a larger fraction of cultured cells to synthesize DNA than could pork insulin or two of the insulin-like growth factors, MSA and IGF-I. In addition, when maximal concentrations of guinea pig insulin were mixed with either pork insulin or IGF-I, the effect produced was additive which was in contrast to the situation when pork insulin and insulin-like growth factor were mixed; no additivity was observed. These data suggest very strongly that guinea pig insulin and pork insulin mediate their growth effects through distinct pathways including distinct receptors. As a follow-up, ^{125}I-guinea pig insulin was used to give a direct demonstration of a separate and distinct receptor for guinea pig insulin on the human fibroblast.

Biosynthetic Labeling of the Insulin Receptor: The insulin receptor is a cell surface glycoprotein. Receptors on cultured human lymphocytes were labeled biosynthetically with radioactive amino acids and with radioactive sugars. In addition, the protein and carbohydrate components of the receptor were externally labeled with radioactive iodine or, sugars, respectively. The receptor was then solubilized and purified multi-fold by several methods including use of anti-receptor antibodies derived from individual patients. Major subunits detected have molecular weights of 135,000 and 95,000. These same subunits could be labeled by affinity cross-linking techniques. These methods also allow for the measurement of turnover of receptor. Normally the receptor on these cells has a half-life of 9 to 12 hours, and when exposed to insulin, which down-regulates the receptor, the degradation rate is increased by 3 to 4 fold.

Insulin Receptors in Normal Fibroblasts and Fatty Fibroblasts: Using 3T3-L1 cells, which are fatty fibroblasts, it can be shown that glucocorticoids induce the same kinds of alterations that have previously been shown in rats treated with glucocorticoids *in vivo*. This provides the most satisfactory culture model for that *in vivo* condition of insulin resistance.

The functional properties of membrane proteins are dependent on the lipid composition of the membranes in which they reside. It was shown in these cells that the membrane lipids can be directly altered by exposure to other lipids, which in turn alters the function of the insulin receptor.

Extreme Insulin Resistance

Insulin Binding and Insulin Action in Cultured Human Fibroblasts: One major group of patients who have severe resistance to the effects of insulin appear to have inborn errors localized to the receptor or other early steps in the action of insulin at the target cell. Fibroblasts from individual patients provide an opportunity to study the genetic makeup of these patients. Three clinical syndromes that fall into this major group were studied, i.e., Type A syndrome with acanthosis nigricans, leprechaunism, and lipoatrophic diabetes. In some of the patients insulin binding to fibroblasts was normal whereas in others there were defects in the affinity of the receptor for hormone or decreases in receptor concentration or modest abnormalities in the characteristics of the receptor. More extensive characterization is now underway.

To measure insulin effects in these cells, studies were made of glycogen synthase which is active in its dephosphorylated form and less active in its phosphorylated form. With cells from individual patients, correlations were made between enzyme activation and hormone binding in an attempt to localize the sites of the defects in insulin action.

Another charactistic effect of insulin is to decrease the concentration of its own receptors ("down-regulation"). Insulin receptors on human fibroblasts down regulate in response to chronic exposure to insulin. In most cases this is due to a decrease in receptor concentration but conditions can be set to produce alterations in receptor affinity. Most of the patients were capable of responding to insulin with a decrease in receptor concentration but a few patients were not. In particular, patients who appeared to have post-receptor defects demonstrated these failures in down-regulation.

Studies of B Lymphocyte Cell Lines From Patients with Extreme Insulin Resistance: To confirm and extend data provided by the fibroblasts, techniques to produce continuous cell lines from lymphocytes of individual patients have been introduced. Two patients with extreme insulin resistance, one with Type A and one with leprechaunism, were both shown to have a decrease in the concentration of the insulin receptors which correlates well with the clinical findings and results of other studies in these patients.

In another study with cultured lymphocytes from a patient with leprechaunism, an abnormal receptor was detected; a major characteristic of this receptor was that insulin binding is exceptionally insensitive to changes in temperature and pH. In addition to the studies described, multiple new techniques are being added to characterize the receptor on these cells which we expect to become a major tool for studies of inherited disorders related to insulin action.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47,003 - 06 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Insulin Receptors and Insulin Responsiveness in 3T3-L1 Fatty Fibroblasts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	C. R. Kahn	Senior Surgeon	DB, NIADDK
Other:	K. L. Baird	Biologist	DB, NIADDK
	C. Grunfeld	Assist Prof of Med	Univ California
	C.-C. Wang	Guest Worker	DB, NIADDK
	M. Crettaz	Guest Worker	DB, NIADDK

COOPERATING UNITS (if any)

Department of Medicine, University of California, San Francisco, CA

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Insulin receptors and responsiveness have been studied in undifferentiated and fatty 3T3-L1fibroblasts. Glucocorticoid hormones induce insulin resistance in 3T3-L1 cells. The affinity of the receptor for insulin is decreased and there is inhibition of the insulin response at a post-receptor site. Membrane lipids can be modified by growth of cells in media containing different saturated and unsaturated fatty acids. Saturated fatty acids result in a decrease in both insulin binding and action.

PHS-6040
(Rev. 10-76)

Project Description:

3T3-L1 fibroblasts differentiate into a fatty phenotype which has insulin stimulated glucose transport, glucose oxidation, lipoprotein lipase and fatty acid and triglyceride synthesis, as well as insulin inhibited lipolysis. We have previously used this insulin-responsive cultured cell line to explore the effects of antibody against the insulin receptor on a long-term insulin action, and to study morphometrically the interaction of insulin with fat cells.

Glucocorticoid hormone excess causes insulin resistance in man and animals. It has not been possible to induce these changes in vitro using freshly isolated adipocytes. Exposure of 3T3-L1 cells to physiologic and pharmacologic concentrations of glucocorticoid hormones for 2-4 days produces insulin resistance. The affinity of the receptor for insulin is reduced throughout occupancy. Maximal insulin stimulation of glucose uptake is dramatically reduced. Basal uptake is slightly reduced. The cells are also less sensitive to insulin; half-maximal stimulation occurs at a 10-fold higher concentration of insulin. Since the affinity of the receptor is shifted only three-fold, this provides evidence that glucocorticoid hormones exert their effects at post-receptor steps as well as affecting the insulin receptor.

It has previously been shown that feeding fatty acids to cultured cells grown with dialyzed fetal calf serum results in extensive modification of phospholipid fatty acid composition (Spector et al., J. Lipid Res. 20: 536, 1979). We have used this technique to determine whether such changes influence the response to insulin in differentiated 3T3-L1 fatty fibroblasts, a cultured fat cell line.

A 50-60% decrease in specific insulin binding was seen when 3T3-L1 cells were fed with media containing the saturated fatty acids, myristic, palmitic and stearic acid. By contrast, small decreases in insulin binding were seen when cells were fed the unsaturated analogues of similar chain length, myristoleic, palmitoleic and oleic acids. This decrease in insulin binding was due to a decrease in receptor number.

Fatty acid feeding resulted in inhibition of insulin action as measured by a decrease in the maximal stimulation of deoxyglucose uptake by insulin with little change in sensitivity to insulin. Saturated fatty acids also affected insulin action more profoundly than unsaturated analogues of similar chain length, with palmitic acid showing more than 80% inhibition. A significant correlation ($R = 0.881$, $p < 0.01$) was found between the inhibition of insulin binding and the decrease in insulin-stimulated deoxyglucose uptake, despite the presence of spare receptors.

Thus, modification of cellular lipids affects both the exposure of insulin receptors and the ability of insulin to exert its biological effects.

Publications:

1. Grunfeld, C., Baird, K. L., Van Obberghen, E., and Kahn, C. R.: Glucocorticoid-induced insulin resistance in vitro. Evidence for both receptor and post-receptor defects. Endocrinol., in press.

Awards:

C. R. Kahn is the recipient of the Eli Lilly Award for Diabetes Research given by the American Diabetes Association, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47,005 - 09 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Insulin Binding in Circulating Cells of Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	I. G. Fantus	Visiting Fellow	DB, NIADDK
	R. F. Dons	Clinical Associate	DB, NIADDK
	G. A. Saviolakis	Staff Fellow	DB, NIADDK
	G. Grunberger	Clinical Associate	DB, NIADDK
	C. M. Hendricks	Bio Lab Tech	DB, NIADDK
	P. Gorden	Medical Director	DB, NIADDK
	N. Hizuka	Visiting Associate	DB, NIADDK
	J. Roth	Medical Director	DB, NIADDK
Others:	L. M. Corash	Senior Surgeon	CP, CC
	R. S. Bar	Associate Professor	Univ of Iowa
	M. Muggeo	Prof of Endocrinology	Univ of Ancona

COOPERATING UNITS (if any)
Hematology Service, Clinical Pathology Branch, Clinical Center; Department of Internal Medicine, University of Iowa, Iowa City, Iowa; Department of Endocrinology, University of Ancona, Ancona, Italy

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section and Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.4	2.9	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Numerous studies from this laboratory have investigated the interaction of insulin with circulating mononuclear cells. It has been demonstrated that obese diabetic individuals have a deficiency in insulin receptors and these are reversed by appropriate diet. Further, numerous other diseases of receptor deficiency have been identified. The present project involves studies on in vivo and in vitro states of insulin-dependent diabetes mellitus (IDDM) and glucocorticoid excess. We have attempted to characterize the insulin receptor of the human erythrocyte.

Project Description:

We have studied a group of patients with insulin-dependent diabetes mellitus and have observed that insulin binding is either normal or slightly elevated. This is consistent with the severe hypoinsulinemia seen in these individuals and consictent with the animal models of insulin dependent diabetes. In addition, conditions which are seen in patients with diabetes were simulated in vitro, i.e. ketoacidosis, hyperglycemia, and acidemia. While these factors may influence binding in the patient they are not perpetuated into the in vitro assay; thus there is no "memory" for these abnormalities when taken from the patient and studied in vitro. By contrast, starvation and hyperinsulinemia are conditions for which the receptor has "memory" and the abnormalities are perpetuated into the in vitro assay. Corticosteroids in vitro increased insulin binding but in normal individuals who also develop hyperinsulinemia in response to corticosteroids we have been unable to demonstrate this effect. In a series of studies it has been shown that corticosteroids definitely influence insulin binding through the corticosteroid receptor on cultured human lymphocytes.

In an attempt to understand the effects of glucocorticoids on the insulin receptor, we have studied this phenomenon both in vitro and in vivo. In vitro preincubation with hydrocortisone in the cultured human lymphocyte leads to an increase in the number of insulin receptors. By contrast there is a decrease in the number of growth hormone receptors on this cell. To our knowledge this is the first demonstration of the induction of insulin receptors by a heterotrophic agent. When the effect of prednisone was tested in normal volunteers we find that the administration of 40 mg of prednisone per day produces no statistically significant difference in either tracer insulin binding, the number of insulin receptors, or the affinity of the insulin receptor. The explanation for these findings is not completely clear but a tentative hypothesis is that in blood cells, at least, glucocorticoids probably cause an increase in the number of insulin receptors as reflected by our in vitro studies. However, in vivo when the concentration of circulating insulin rises concomitantly, there is an opposing down regulation of the insulin receptor. Thus, at least under the conditions studied, no net change in insulin receptors are seen. These findings are in contrast with the effect of glucocorticoids on other target cells such as adipocytes and hepatocytes.

In a separate set of studies in patients with the Type A form of insulin resistance and the Type B form of insulin resistance, i.e. that form associated with anti-insulin receptor antibodies, we have characterized extensively the nature of binding effect in individual patients. In the Type A syndrome we find a decrease in the number of insulin receptors on circulating monocytes while in the Type B syndrome we find a major alteration in the affinity of insulin binding to these cells. This appears to be a relatively uniform finding in the two different groups of patients.

In a separate series of studies we have shown that human erythrocytes have specific insulin receptors and that the concentration of these receptors on the cell surface is exponentially related to cell age. Thus, red blood

Project Description:

cells lose receptors as an exponential function of their cell age and this is independent of the reticulocyte content of the preparation. This may be the first example of an age related integral protein of the red blood cell membrane.

Publications:

1. Bar, R. S., Muggeo, M., Kahn, C. R., Gorden, P., and Roth, J.: Characterization of insulin receptors in patients with the syndromes of insulin resistance and acanthosis nigricans. Diabetologia 18: 209-216, 1980.

2. Dons, R. F., Corash, L. M., and Gorden, P.: The insulin receptor is an age-dependent integral component of the human erythrocyte membrane. J. Biol. Chem. 256: 2982-2987, 1981.

3. Fantus, I. G., Ryan, J., and Gorden, P.: The insulin receptor in insulin-dependent diabetes mellitus: An *in vivo* and *in vitro* study. Metabolism 30: 510-517, 1981.

4. Fantus, I. G., Ryan, J., Hizuka, N., and Gorden, P.: The effect of glucocorticoids on the insulin receptor: An *in vivo* and *in vitro* study. J. Clin. Endocrinol. Metab. 52: 953-960, 1981.

5. Wachslicht-Rodbard, H., Muggeo, M., Kahn, C. R., Saviolakis, G. A., Harrison, L. C., and Flier, J. S.: Heterogeneity of the insulin receptor interaction in lipoatrophic diabetes. J. Clin. Endocrinol. Metab. 52: 416-425, 1981.

6. Roth, J., and Muggeo, M.: Insulin receptors and hyperglycemia. *In* Rifkin, H., and Raskin, P. (eds.): Diabetes Mellitus, volume V, Robert J. Brady Company, Bowie, Maryland, 1981, pp. 63-77.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47,007 - 06 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Antibodies to Receptors: Detection in Disease States and Use as Probes to Study Receptor Function and Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	S. I. Taylor	Surgeon	DB, NIADDK
	L. H. Underhill	Biologist	DB, NIADDK
	J. Roth	Medical Director	DB, NIADDK
	P. Gorden	Medical Director	DB, NIADDK
Other:	T. Waldman	Medical Director	MET, NCI
	R. M. Pruss	Staff Fellow	LCS, NIMH

COOPERATING UNITS (if any)
Metabolism Branch, National Cancer Institute; Laboratory of Clinical Science, National Institute of Mental Health

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.9	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using assays based on the ability of anti-receptor antibodies to immunoprecipitate insulin receptors to inhibit insulin binding, we have screened patient sera for the presence of anti-receptor antibodies. These anti-receptor antibodies are extraordinarily useful reagents for biosynthetic studies of insulin receptors. We are attempting to produce monoclonal antibodies directed against the receptor.

PHS-6040

Project Number: Z01 AM 47,007 - 06 DB

Project Description:

The interaction of insulin receptor antibodies with the solubilized insulin receptor has been studied in detail. Solubilized insulin receptors were prepared by extracting human placental membranes with Triton X-100, and assaying for ^{125}I-insulin binding activity using polyethylene glycol to precipitate receptors. These solubilized receptors retain all the properties, including negative cooperativity, of membrane-bound receptors. Receptor antibodies inhibited insulin binding to the solubilized receptors with the same titers as previously reported using whole cells. This inhibition was due to a decrease in the number of available insulin binding sites rather than a decrease in their affinity as seen with membrane-bound receptors. We are using this assay to screen sera from patients for the presence of anti-insulin receptor antibodies. These anti-receptor antibodies are useful agents in the studies of the structure of the insulin receptor.

In addition to the above studies we are currently trying to prepare a monoclonal antibody to the insulin receptor. Partially-purified human placental insulin receptors were used to immunize Balb/C mice. After 4 days the mice spleen cells were hybridized to the P3 x 63 line of mouse myeloma cells (kindly donated by Dr. G. Eisenbarth). Several hybridoma lines grew out which appeared by several criteria to be secreting receptor antibodies. These have been passaged as ascites tumors in Balb/C mice prior to cloning.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47,010 - 09 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Patient Studies Using MSA (Multiplication Stimulating Activity) Radioreceptor Assay.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	C. M. Hendricks	Bio Lab Tech	DB, NIADDK
	P. Gorden	Medical Director	DB, NIADDK
	J. Roth	Medical Director	DB, NIADDK
	C. R. Kahn	Senior Surgeon	DB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.2	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

MSA (Multiplication Stimulating Activity) is an insulin-like peptide which reacts with its own receptor and the insulin receptor. MSA is elevated in about 40% of patients with hypoglycemia and non-islet cell tumors. Patients with hemangiopericytoma have elevated circulating MSA levels.

PHS-6040

Project Number: Z01 AM 47,010 - 09 DB

Project Description:

This study was originally designed to study the physiology and pathophysiology of circulating growth factors in man. When a crude NSILA-s preparation or a highly purified multiplication stimulating activity preparation was incubated with purified liver membranes, a highly sensitive assay for these growth peptides was developed. The most recent aspect of this study is an attempt to characterize those patients who are most likely to demonstrate an elevation of an MSA cross-reacting material. Thus, in a study of over 52 patients with tumor-induced hypoglycemia of a non-insulin nature we have found that 6 of 7 patients with hemangiopericytoma have elevations of the circulating growth factor-like material. Since these materials are insulin-like and since in a single patient we have shown that the level falls as the patient goes into remission, we feel that this circulating insulin-like material is, at least in part, responsible for the hypoglycemia seen in these patients.

When plasma is filtered over a G-75 Sephadex column that is developed in and eluted by 1M acetic acid we find a peak eluting in the region of the MSA marker. In addition, we find a peak eluting approximately of double the molecular size of MSA, i.e. a peak that is more retarded by the gel. The nature of this peak is uncertain at the present time, investigations are under way to determine whether this might be a form of a binding protein. Preliminary evidence suggests that some of the patients with tumor hypoglycemia may have deficiency in binding protein. If this is the case, then we are looking at the minimum number of elevated samples. Thus, the effective dose of the growth factor would be the free concentration. If the binding protein was deficient the free concentration would undoubtedly be elevated.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47,014 - 12 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Acromegaly

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	F. de Pablo	Visiting Fellow	DB, NIADDK
	J. Roth	Medical Director	DB, NIADDK
Other:	P. Gorden	Medical Director	DB, NIADDK
	R. C. Eastman	Investigator	Maine Medical Center, Portland, Maine

COOPERATING UNITS (if any)
Maine Medical Center (Department of Internal Medicine), Portland, Maine

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The standard therapy for growth hormone elevation in acromegaly has been conventional supervoltage irradiation. The data of human prolactin levels in acromegalics who received radiotherapy has been elevated at 2, 5, and 10 year follow-ups. The data suggest a correlation between prolactin and human growth hormone not only at the level of pituitary secretion but possibly also at the target cell.

Project Number: Z01 AM 47,014 - 12 DB

Project Description:

Plasma prolactin (hPRL) was measured in seventy-three untreated acromegalic patients, and found to be elevated in 32% of the total population Hyperprolactinemia was present in 40% of the females and in 27% of the male patients. In both groups plasma hRPL correlated with plasma hGH levels with correlation coefficients of 0.38 ($p < 0.05$) for females and 0.41 ($p < 0.005$) for males. Forty-five patients were treated with conventional supervoltage pituitary irradiation and evaluated at 2, 5, and 10 years after treatment. The patients with hyperprolactinemia before irradiation showed a decrease in plasma hPRL at the most recent follow up, (mean ± SEM, 125 ± 34 ng/ml versus 67 ± 15 ng/ml, $p < 0.01$) although, in general, they did not achieve normal values. The patients who had normal plasma hPRL before irradiation (mean ± SEM, 14 ± 2 ng/ml) had an increase in their hPRL levels after therapy (23 ± 3 ng/ml, $p < 0.005$) but remained in the normal range during long term follow-up. In ten patients followed for 1-10 years without treatment, there was a tendency for the plasma hPRL to rise progressively (mean increment 122% above the initial value) with individual changes in hPRL strikingly parallel to the changes in plasma hGH. When serum hPRL was initially elevated similar responses in both hormones were also seen in a small group of patients treated with surgical hypophysectomy. Galactorrhea was present in five of the twenty-five female patients; in 4 of the 5, plasma prolactin was within the normal range. Overall these data suggest a closer relationship between hGH and hPRL in acromegaly than had been suspected, not only at the level of pituitary secretion but possibly also at the target cell.

Publications:

1. De Pablo, F., Eastman, R. C., Roth, J., and Gorden, P.: Plasma prolactin in acromegaly before and after treatment. J. Clin. Endocrinol. Metab., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47,016 - 05 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Structure of the Insulin Receptor Studied by Ionizing Radiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. T. Harmon	Guest Worker	DB, NIADDK
	C. R. Kahn	Senior Surgeon	DB, NIADDK
Other:	E. S. Kempner	Senior Investigator	LPB, NIADDK

COOPERATING UNITS (if any)

Laboratory of Physical Biology, NIADDK

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The results of radiation inactivation studies have indicated that the insulin receptor in rat liver membranes is composed of at least two components: an affinity-regulatory component with an apparent molecular weight of ∿300,000 and a binding component with a molecular weight of ∿100,000. The affinity-regulator acts as an inhibitor of insulin binding by decreasing the affinity of the binding component for insulin. Conditions which alter the affinity of the receptor (pH and ionic strength) also alter the inactivation profile. The higher the affinity of the receptor, the larger the proportion of binding components which are not associated with affinity-regulators. The interaction between the affinity-regulator and binding component are reversible suggesting that these components are not covalently bound. The affinity-regulator is a trypsin-sensitive membrane protein which can be separated from the binding component by lectin-affinity chromatography. Insulin bound to the receptor causes a change in the size of the binding component from 115,000 to 76,000 with no change in the apparent size of the affinity regulator.

Project Number: Z01 AM 47,016 - 05 DB

Project Description:

Using radiation inactivation, we have previously shown that the insulin receptor in situ in liver membranes contains two functional components, a binding component with an apparent molecular weight of ∿100,000 and an affinity-regulatory component of ∿300,000. The affinity-regulator interacts with the binding component causing a decrease in receptor affinity; because of its larger size, it is destroyed at lower radiation doses with a resultant increase in receptor affinity.

We have used the technique of radiation inactivation to examine whether insulin alters the size or structure of the insulin receptor. When insulin is bound to its receptor in liver membranes before radiation, there is a marked change in the inactivation profile. Analysis of the results indicated that insulin binding to its receptor caused a decrease in the functional size of the insulin binding component from 115,000 to 76,000 and also diminished the interaction between the binding and regulatory components. The magnitude of these effects depended on the insulin concentration, and analogues of insulin with reduced receptor affinity show a reduced effect. Under these conditions, there was no change in the apparent size of the affinity regulator (about 260,000). These data suggest that the interaction of insulin with its receptor in situ produces a change in receptor structure which may be important in signal transmission.

To better understand the nature of the affinity-regulatory and its interaction with the binding component we have examined the insulin receptor under circumstances in which the affinity of the receptor was altered by varying the pH of the binding assay or by increasing the ionic strength. Under circumstances in which the affinity of the receptor was increased, the interaction between the regulatory and binding components was either decreased or absent, i.e. there was no increase in binding with irradiation. Conversely, decreasing receptor affinity resulted in an increase in the interaction between the regulatory and binding components. In both cases, the changes in affinity and interaction of the two components was reversible. By contrast, in membranes of ob/ob mice in which binding was altered by changing receptor number, there was no detectable change in the interaction of the two receptor components. Under no condition did the size of either component vary. In an attempt to characterize the nature of the affinity-regulator, liver membranes were treated with varying concentrations of trypsin prior to irradiation. Low concentrations of trypsin (1-5 μg/ml) and low doses of radiation had very similar effects i.e. both increased insulin receptor affinity. Furthermore, after trypsin treatment the effect of radiation on receptor affinity was lost. Filtration of the solubilized receptor on lectin-affinity columns also resulted in the apparent removal of the affinity-regulator, i.e. receptor affinity was increased and radiation produced no further change. These data suggest that (1) variations in pH and ionic strength which modulate receptor affinity alter the interaction of the regulatory and binding components; (2) the interaction is reversible suggesting that these two components of the receptor are not covalently linked; and (3) the affinity-regulatory is a trypsin sensitive membrane protein which can be separated from the binding component by lectin-affinity chromatography.

Publications:

1. Harmon, J. T., Kempner, E. S., and Kahn, C. R.: Demonstration by radiation inactivation that insulin alters the structure of the insulin receptor in rat liver membranes. J. Biol. Chem., in press.

2. Harmon, J. T., Kahn, C. R., Kempner, E. S., and Johnson, M. L.: Examination of the insulin receptor in the membrane by radiation inactivation studies. In Proceedings of the First International Symposium on Insulin Receptors, Rome, Italy, 1980, in press.

3. Kahn, C. R., Baird, K. L., Flier, J. S., Grunfeld, C., Harmon, J. T., Harrison, L. C., Karlsson, F. A., Kasuga, M., King, G. L., Lang, U., Podskalny, J. M., and Van Obberghen, E.: Insulin receptors, receptor antibodies and the mechanism of insulin action. Recent Progress in Hormone Research, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47,018 - 04 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cellular Insulin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. L. Rosenzweig	Clinical Associate	DB, NIADDK
	D. Le Roith	Visiting Scientist	DB, NIADDK
	M. A. Lesniak	Research Chemist	DB, NIADDK
	F. de Pablo	Visiting Fellow	DB, NIADDK
	J. Roth	Medical Director	DB, NIADDK
Others:	D. Shiloach	Visiting Scientist	LNE, NIADDK
	B. Samuels	Research Chemist	DB, NIADDK
	R. Pruss	Staff Fellow	LCS, NIMH

COOPERATING UNITS (if any)
Laboratory of Nutrition and Endocrinology, NIADDK
Laboratory of Clinical Science, National Institute of Mental Health

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An insulin-like material characterized by radioimmunoassay, gel filtration and bioactivity in fat cells can be found in eukaryote and prokaryote organisms. In addition to insulin a few other hormones - ACTH and somatostatin have been characterized in unicellular organisms. These findings suggest that the origins of the endocrine system appeared earlier than previously thought. We are also continuing the studies on extrapancreatic insulin found in the guinea pig.

Project Number: Z01 47,018 - 04 DB

Project Description:

To further substantiate that the pancreas is not essential for the production of insulin, two invertebrate species were studied. Acid-ethanol extracts of Drosophila melanogaster and earthworms contained material very similar to mammalian insulin with respect to the gel filtration pattern, reactivity in the radioimmunoassay and bioassay for classic insulins. The bioactivity could be neutralized by anti-insulin antibody. Furthermore, the bio:immuno ratio was similar to mammalian insulins.

Similar insulin-like material was demonstrated in extracts of unicellular eukaryotes (tetrahymena pyriformis, a citiated protozoan, and two fungi, neurospora crassa and aspergillus fumigatus) as well as in the prokaryote E. Coli K-12, which had been grown in defined medium free of added macromolecules or serum.

In addition to insulin a number of other classic vertebrate hormones have been characterized in the unicellular organisms. Both ACTH and somatostatin have been demonstrated with immunologic, biologic and chromatographic similarities to classic synthetic ACTH (1-39) and synthetic somatostatin.

These findings suggest that the origins of the endocrine system is much earlier than previous thought, and that most cells are capable of making most, if not all, peptide hormones.

We have previously published data showing that guinea pigs have more than one type of insulin (characterized by classical methods - see above description). One synthesized and secreted by the pancreas into plasma and a second insulin native to extrapancreatic tissues. These data suggest that insulin bound in extrapancreatic tissues is synthesized locally rather than in the pancreas. In support of this thesis we are continuing studies on the synthesis of insulin (and other hormones) in other vertebrates such as chick embryos prior to the development of the organ, e.g. pancreas.

Publications:

1. Le Roith, D., Shiloach, J., Roth, J., and Lesniak, M. A.: Evolutionary origins of vertebrate hormones: Substances similar to mammalian insulins are native to unicellular eukaryotes. Proc. Natl. Acad. Sci., USA 77: 6184-6188, 1980.

2. Rosenzweig, J. L., Lesniak, M. A., Samuels, B. E., Yip, C. C., Zimmerman, A. E., and Roth, J.: Insulin in the extrapancreatic tissues of guinea pigs differ markedly from the insulin in their pancreas and plasma. Trans. Assoc. Am. Phys. 93: 263-278, 1980.

3. Le Roith, D., Lesniak, M. A., and Roth, J.: Insulin in insects and annelids. Diabetes 30: 70-76, 1981.

Publications Continued:

4. Lesniak, M. A., and Roth, J.: Insulin and growth hormone: Interactions with specific receptors on the cell surface. In Erberle, A., Geiger, R., and Wieland, T., (eds.): Perspectives in Peptide Chemistry, S. Karger A. G., Basal, Switzerland, 1981, pp. 395-406.

5. Rosenzweig, J. L., Havrankova, J., Brownstein, M., and Roth, J.: Insulin in the brain and other extrapancreatic tissues. In Stark, E., Makara, G. B., Acs, Zs., and Endröczi, E. (eds.): Advances in Physiological Science, Volume 13, Pergamon Press, Budapest, 1981, pp. 175-186.

6. Van Obberghen, E., De Pablo, F., and Roth, J.: Insulin receptors in disorders of glucose tolerance and insulin sensitivity. In Lefkowitz, R. J., (ed.): Receptors and Recognition, series B, Volume 13, Chapman and Hall, London, 1981, in press.

7. Van Obberghen, E., and Roth, J.: The insulin receptor and its function. In Lefkowitz, R. J. (ed.): Receptors and Recognition, series B, Volume 13, Chapman and Hall, London, 1981, in press.

8. Underhill, L., Rosenzweig, J. L., Roth, J., Brownstein, M., Young, W. S., and Havrankova, J.: Insulin and insulin receptors in the nervous system of mammals. Frontiers of Hormone Research, 1981, in press.

9. De Pablo, F., Roth, J., and Hedo, J.: La insulina y sus receptores celulares en las alteraciones del metabolismo de la glucosa. Rev. Clin. Esp., 1981, in press.

Awards:

J. L. Rosenzweig is the First Fellow supported by the Lita Annenberg Hazen Award for Clinical Research.

J. Roth is the recipient of the following awards:

Herbert McLean Evans Memorial Lecturer, University of California, San Francisco, 1980.

Gairdner Foundation Annual Award, Toronto, Canada, 1980.

A. Cressy Morrison Award in Natural Sciences given by the Board of Governors of the New York Academy of Sciences, 1980.

William H. Creasy Visiting Professorship of Clinical Pharmacology, given by the Burroughs Wellcome Fund, 1981.

Joslin Medal, given by the American Diabetes Association New England Affiliate, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47,019 - 04 DB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Morphological Studies of Ligand Binding to Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	P. Gorden	Medical Director	DB, NIADDK
	J.-L. Carpentier	Chargé de Recherches	Univ of Geneva
	E. Van Obberghen	Visiting Associate	DB, NIADDK
	M. A. Lesniak	Research Chemist	DB, NIADDK
	N. Hizuka	Visiting Associate	DB, NIADDK
Other:	L. Orci	Prof of Medicine	Univ of Geneva
	P. Freychet	Prof of Medicine	Univ of Nice
	A. Le Cam	Chargé de Recherches	Univ of Nice
	P. Barazzone	Research Associate	Univ of Geneva

COOPERATING UNITS (if any)

Institute of Histology and Embryology, University of Geneva, Geneva, Switzerland; Departments of Medicine and Biochemistry, University of Nice, Nice, France

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

^{125}I-insulin binds to IM-9 cells (human) and hepatocytes (rat). By using quantitative EM autoradiography as a probe, it can be shown that labeled insulin is internalized by a process which appears to be time- and temperature-dependent. Other labeled proteins (low density lipoprotein, epidermal growth factor, human growth hormone, glucagon, and anti-insulin receptor antibody) appear to behave in the same fashion i.e. initial binding followed by internalization.

Project Number: Z01 AM 47,019 - 04 DB

Project Description:

This project represents a group of continuing studies of the visualization of polypeptide hormones and other ligands as they interact with target cells. We used quantitative EM autoradiography as our visual probe and have demonstrated that polypeptide hormones initially bind to cell surfaces and are subsequently internalized by the cell as a function of time and temperature. In the present group of studies we find 1) that when labeled insulin binds to IM-9 cultured lymphocytes at low temperatures the binding is random and occurs with equal frequency at each pole of the cell. This cell has a polarity that is marked by the location of the Golgi region, i.e. it can be divided between a Golgi and non-Golgi pole. Initially binding is random at either pole but as a function of temperature, primarily, and to some extent, time, the ligand is subsequently redistributed towards the Golgi pole. This suggests that the ligand is mobile in the plane of the membrane. 2) Glucagon is another polypeptide hormone that has multiple effects on hepatocytes. In the present study we have shown that ^{125}I-glucagon binds to the surface of the hepatocytes and is subsequently internalized as a function of time and temperature. Glucagon, qualitatively, is internalized in a manner similar to insulin but quantitatively there is a lag over the first 20 minutes of incubation at 37°C, and then a build-up so that by 30-60 minutes approximately 30% of the ligand is internalized and approximately 70% remains on the plasma membrane. Lysosomal inhibitors inhibit glucagon degradation but the material bound to the cell is largely intact. The material dissociated from the cell is a function of incubation time at 37°C is largely degraded. 3) ^{125}I-growth hormone also binds to IM-9 cultured lymphocytes in a specific and saturable manner. When this ligand is incubated at low temperatures with cultured lymphocytes ^{125}I-hGH binds to the cell surface. Again, as with the other polypeptide hormones, hGH is internalized in a time and temperature dependent fashion. Growth hormone, in contrast to insulin, is only partially dissociable from the cell. When quantitative EM autoradiography is carried out under dissociation conditions, it is clear that the non-dissociability of the ligand is related to both internalized ligand and to the ligand on the cell surface. Thus, the growth hormone system demonstrates several phenomenon that may be important with respect to ligand-induced receptor regulation, i.e., there is a irreversible binding step which occupies receptors irreversibly. In addition there is a internalization step in which the ligand and presumably the receptor are internalized and there is a step intracellularly involving non-dissociability of the ligand once it has been internalized by the cell. 4) Because of the findings in the growth hormone system of non-dissociability, we further studied the effects of lysosomal inhibitors on this system. We find that lysosomal inhibitors increased the growth hormone binding to the cell or more correctly stated, increased cell-associated radioactivity when incubations were carried out at 37°C. There is little effect of these agents at lower temperatures. Thus, the lysosomal inhibitors appear to inhibit degradation of growth hormone, strongly suggesting that degradation is at least in part a lysosomal function. In addition because of the sensitivity of down regulation of the growth hormone system, we have been able to show directly that at equal concentrations of hormone, i.e.,

Project Description:

concentrations that can be used as tracers for the morphologic studies and concentration used to down regulate the cells, that there is a direct correlation between the proportion of ligand internalized and that bound to the cell, i.e. internalization and down regulation are directly correlated. These studies provide support for a concept that ligand-induced receptor regulation is linked to the receptor by the internalization step.

Publications:

1. Gorden, P., Carpentier, J.-L., Freychet, P., and Orci, L.: Internalization of polypeptide hormones: mechanism, intracellular localization and significance. Diabetologia 18: 263-274, 1980.

2. Carpentier, J.-L., Gorden, P., Freychet, P., and Orci, L.: Aspects morphologiques de l'interaction de l'insuline avec sa cellule cible. Journées Annuelles de Diabétologie de l'Hôtel Dieu, 1980, pp. 276-282.

3. Barazzone, P., Carpentier, J.-L., Gorden, P., Van Obberghen, E., and Orci, L.: Polar redistribution of ^{125}-iodine-labeled insulin on the plasma membrane of cultured human lymphocytes. Nature 286: 401-403, 1980.

4. Gorden, P., Carpentier, J.-L., Freychet, P., and Orci, L.: Morphologic probes of polypeptide hormone receptor interactions. J. Histochem. Cytochem. 28: 811-817, 1980.

5. Barazzone, P., Gorden, P., Carpentier, J.-L., Freychet, P., Canivet, B., and Orci, L.: Binding, internalization, and lysosomal association of ^{125}I-glucagon in isolated rat hepatocytes: A quantitative electron microscope autoradiographic study. J. Clin. Invest. 66: 1081-1093, 1980.

6. Barazzone, P., Lesniak, M. A., Gorden, P., Van Obberghen, E., Carpentier, J.-L., and Orci, L.: Binding, internalization and lysosomal association of ^{125}I-human growth hormone in cultured human lymphocytes: A quantitative morphologic and biochemical study. J. Cell Biol. 87: 360-369, 1980.

7. Hizuka, N., Gorden, P., Lesniak, M. A., Van Obberghen, E., Carpentier, J.-L., and Orci, L.: Polypeptide hormone degradation and receptor regulation are coupled to ligand internalization. J. Biol. Chem. 256: 4591-4597, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47,021 - 03 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Insulin Receptors and Action in Cultured Human Fibroblasts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	C. R. Kahn	Senior Surgeon	DB, NIADDK
	J. M. Podskalny	Bio Lab Tech	DB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Insulin binding and action have been studied in cultured human fibroblasts. Cells are derived from controls, insulin resistant patients, and diabetics. Activation of glycogen synthase and induction down-regulation, are being examined in these cells. Insulin receptor defects have been found on approximately half of the cell lines from insulin resistant patients. These include defects in receptor number, receptor affinity, and pH optimum of binding. Post-receptor defects, in the presence of normal insulin receptors, are also present in some cells from insulin resistant patients. Cells from Type I diabetics are normal in most respects.

PHS-6040
(Rev. 10-76)

Project Number: Z01 AM 47,021 - 03 DB

Project Description:

We are examining insulin binding and insulin action in cultured fibroblasts from patients with Type I diabetes and patients with a variety of states of severe insulin resistance. The insulin resistant states in which we are particularly interested are the Type A syndrome of insulin resistance and acanthosis nigricans, and lipoatrophic diabetes. Our principle means of looking at insulin action in these cells is by measuring the activation of glycogen synthase by insulin and by determining the extent and sensitivity of the ability of insulin to "down regulate" its receptors.

In three of our patients with the Type A syndrome of insulin resistance and acanthosis nigricans, insulin binding is reduced. Two cell lines appear to have fewer receptors (decreased R_o), while one has insulin receptors of lowered affinity. This latter situation is also present in cells from an infant with insulin resistance and leprechaunism. These cell lines also show reduced reactivity with antibodies to the insulin receptor. Cell lines from four patients with a variant form of the Type A syndrome were also examined and in two, R_o was decreased. A third variant Type A cell line has only a pH sensitivity defect in its receptor, while the fourth has totally normal binding properties. Cell lines from one patient with total lipodystrophy and from three patients with partial lipodystrophy all had normal insulin binding. Specific binding to cell lines from nine Type I diabetics was normal, although total binding was elevated due to abnormally high levels of non-specific binding in three cell lines.

Insulin stimulates the conversion of the phosphorylated form of glycogen to the dephosphorylated form at concentrations of 1 to 1000 ng/ml in human fibroblasts. The ED_{50} for this effect is between 10 and 50 ng/ml in control cells. The ED_{50} in the Type A cell line with lowered affinity and in the Type A cell line with decreased R_o are slightly elevated. More marked abnormalities in insulin stimulation of glycogen synthase were observed in two cell lines with normal receptors: one from a variant Type A patient and one from a patient with partial lipoatrophic diabetes. The maximal stimulation is not altered in any of these cell lines. Cells from Type I diabetics were totally normal in their response to insulin in the glycogen synthase assay. One patient with early onset diabetes did have abnormally low levels of enzyme, however.

Insulin preincubation can reduce the binding of ^{125}I-insulin to human fibroblasts in a dose related fashion. Up to 60% of the specific binding can be lost by four hours of incubation with 100 ng/ml insulin. This process and also the recovery of receptor sites is dependent upon protein synthesis. If the preincubation with insulin is done in the absence of glucose and serum, the loss of binding capacity appears to be due to a decrease in receptor affinity. If the preincubation is done in conditioned media, R_o appears to be decreased. In either case, the result of down regulation is manifested in the glycogen synthase assay as both a decrease in maximal stimulation and an increase in ED_{50}.

Project Description:

When cells from the insulin resistant patients were examined, all but four cell lines were down regulated with the same concentration of insulin and to the same extent as control cells. Both patients with insulin receptors of decreased affinity were not down regulated except at very high insulin concentrations. Two cell lines from variant Type A patients, both of which had elevated ED_{50} in the glycogen synthase assay, were not sensitive to down regulation.

Receptor, post-receptor, or both defects are thus present in all cell lines derived from patients with both the Type A syndrome of insulin resistance and acanthosis nigricans, and with the variant forms of this disorder. Only one of four cell lines from patients with lipoatrophic diabetes has any abnormalities and these were at the post-receptor level.

Publications:

1. Podskalny, J. M., and Kahn, C. R.: Insulin activates glycogen synthase in cultured human fibroblasts. Diabetes 29: 724-729, 1980.

2. Rechler, M. M., Zapf, J., Nissley, S. P., Froesch, E. R., Moses, A. C., Podskalny, J. M., Schilling, E. E., and Humbel, R. E.: Interaction of insulin-like growth factors I and II and Multiplication Stimulating Activity with receptors and serum carrier proteins. Endocrinol. 107: 1451-1459, 1980.

3. Kahn, C. R., Baird, K. L., Flier, J. S., Grunfeld, G., Harmon, J. T., Harrison, L. C., Karlsson, F. A., Kasuga, M., King, G. L., Lang, U. C., Podskalny, J. M., and Van Obberghen, E.: Insulin receptors, receptor antibodies, and the mechanism of insulin action. Rec. Prog. Horm. Res., in press.

4. Podskalny, J. M., and Kahn, C. R.: Cell culture studies in patients with extreme insulin resistance. I. Receptor defects on cultured fibroblasts. J. Clin. Endocrinol. Metab., in press.

5. Podskalny, J. M., and Kahn, C. R.: Cell culture studies on patients with extreme insulin resistance. II. Abnormal biological responses in cultured fibroblasts. J. Clin. Endocrinol. Metab., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47,022 - 02 DB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Insulin Receptors in Syndromes of Extreme Insulin Resistance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	S. I. Taylor	Research Associate	DB, NIADDK
	J. A. Hedo	Visiting Associate	DB, NIADDK
	M. Kasuga	International Fogarty Fellow	DB, NIADDK
	J. Roth	Medical Director	DB, NIADDK
	R. M. Blizzard	Intergov Visiting Prof	DB, NIADDK
	L. H. Underhill	Bio Lab Tech	DB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Diabetes Branch

SECTION

Receptors and Hormone Action Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	2.2	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

B-lymphocyte cell lines were established using Epstein-Barr virus from peripheral blood of normal volunteers, juvenile-onset diabetics and patients with extreme insulin resistance. Several patients with extreme insulin resistance have been found to have decreased numbers of insulin receptors on cultured lymphocytes. These syndromes have included leprechaunism, Type A extreme insulin resistance, and the Rabson-Mendenhall syndrome. Another patient with leprechaunism has been found to have a normal number of receptors. However, the receptors in this patient have a markedly decreased sensitivity to changes in temperatures and pH.

Project Number: Z01 AM 47,022 - 02 DB

Project Description:

Extreme insulin resistance is a rare syndrome. Among the causes are antibodies to insulin, antibodies to the insulin receptor, defects in insulin binding, and defects in the steps of insulin action subsequent to hormone binding. In our present studies we have concentrated primarily on genetic syndrome defects either in insulin binding or post-binding events. By creating permanent cell lines from patients' cells we hope to obtain a set of tools which will contribute to the elucidation of the poorly understood mechanism of insulin action.

Using Epstein-Barr virus, we have established B-lymphocyte cell lines from peripheral blood of normal volunteers, as well as patients with juvenile-onset diabetes, lipoatrophic diabetes, Type A extreme insulin resistance, Type B extreme insulin resistance, leprechaunism, and the Rabson-Mendenhall syndrome. With the exception of cells from a patient with Type A extreme insulin resistance all other patients' cells had markedly diminished ^{125}I-insulin binding. This resulted from a decreased number of insulin receptors. The residual receptors had normal properties as judged by specificity of hormone binding as well as ability to bind anti-receptor antibody.

In another study using cultured B-lymphocytes from a leprechaun patient in which normal binding with ^{125}I-insulin and normal receptor number were exhibited, we found an abnormal response i.e. sensitivity to changes in temperature and pH. We are pursuing studies in abnormal responses in other patient's cultured B-lymphocytes.

Finally, analyses of the structure of the insuln receptor on cells from leprechaun patients has been studied using polyacrylamide electrophoresis in the presence of SDS. The size of the subunits of these receptors appears to be normal while the number of receptor molecules per cell appears to be reduced.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47,023 - 02 DB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Insulin and Insulin-Like Growth Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G. L. King	Senior Assist Surgeon	DB, NIADDK
	C. R. Kahn	Senior Surgeon	DB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Diabetes Branch

SECTION

Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms of action for the stimulation by insulins (natural and chemically-modified) and insulin-like growth factors were studied. The growth effect was measured by ^{3}[H]-thymidine incorporation into cultured human fibroblasts; the metabolic effect was measured by stimulation of glucose oxidation in rat adipocytes. The relative order of potencies of different insulins diverges when metabolic effects and growth effects are compared. The divergences between two types of insulin action suggest that different domains on the insulin molecule are involved in the expression of these bioeffects.

PHS-6040
(Rev. 10-76)

Project Description:

The mechanism of action for the stimulation of growth by insulin and other growth factors such as multiplication stimulating activity, (MSA), insulin-like growth factors I, IGF-I are being studied and compared to the metabolic activities of these peptide hormones. Growth effect was measured by ^{3}[H]-thymidine incorporation into the DNA of cultured human fibroblasts; the metabolic activity was measured by stimulation of glucose oxidation in rat adipocytes.

Previously, we had demonstrated that the metabolic effects of insulin and IGFs were mediated through the insulin receptor. In these studies, their growth-promoting effects are not mediated by the insulin receptor, but are through another growth receptor. The growth-promoting potencies of various naturally-occurring and chemically-modified insulin were studied. The data revealed that the relative order of potencies between the metabolic and the growth effects of various insulins is strikingly different. This divergence between the two types of insulin actions indicates that different domains on the insulin molecule are responsible for each of these bioeffects. In addition, the growth-promoting effect of insulin appeared to have been more conserved than its metabolic effects.

Of all the insulin tested, only the insulins from the hystricomorph family (i.e. casiragua, coypu, guinea pig, and porcupine) were able to stimulate human fibroblasts to synthesize DNA to a greater extent than any other insulin or IGF.

To understand the mechanism of action of the hystricomorph's insulin we used the technique of autoradiography to measure ^{3}[H]-thymidine incorporation into the DNA of human fibroblasts. We have found that guinea pig insulin was able to stimulate more cells to synthesize DNA than pork insulin, MSA and IGF-I, 30%, 10% and 10%, respectively. In addition, when maximum concentrations of either guinea pig plus pork or guinea pig plus MSA were added, the effect produced was additive in contrast to the combination of pork insulin and IGF's were no additivity were observed. These data support the idea that guinea pig insulin and pork insulin are mediating their growth effects through different mechanisms.

Since guinea pig and pork insulin stimulated different populations of cells to synthesize DNA, we explored the possibility that these cells could be differentiated genetically. Human fibroblasts were cloned and each clone was tested with guinea pig insulin and pork insulin. The results showed that the ability of cells to respond to insulin is not due to genetic differences but it is probably due to some metabolic variations.

Since guinea pig and pork insulin appear to have separate mechanisms to stimulate DNA synthesis, the two insulins may have separate receptors on the cell. Using ^{125}I-guinea pig insulin, a separate and distinct receptor for guinea pig has been demonstrated on the human fibroblast that has no or very low affinity for pork insulin.

Project Number: Z01 AM 47,023 - 02 DB

Publications:

1. King, G. L., and Kahn, C. R.: Differential potencies of insulin in metabolic and growth-promoting effects. Nature, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47,024 - 02 DB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biosynthetic Labeling of the Insulin Receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	M. Kasuga	Guest Worker	DB, NIADDK
	J. A. Hedo	Visiting Associate	DB, NIADDK
	C. R. Kahn	Senior Surgeon	DB, NIADDK
	E. Van Obberghen	Guest Worker	DB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section and Receptors and Hormone Action Sec.

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The insulin receptor of cultured human lymphocytes has been characterized by biosynthetic labeling with radioactive amino acids (methionine and leucine) and sugars (fucose, galactose, glucosamine and mannose). In addition the subunits of the receptor have been labeled by cell surface methodology that reveal proteins (lactoperoxidase/$Na^{125}I$) or glycoproteins (galactose oxidase/NaB^3H_4, periodate/NaB^3H_4). The receptor has, as shown by all these methods, two major subunits of molecular weights 135,000 and 95,000 and a minor component of 205,000-210,000. The two major subunits are also revealed by affinity cross-linking with ^{125}I-insulin. The interaction of the solubilized insulin receptor with twelve different lectins have also been analyzed. Using these labeling techniques we have measured directly the turnover rate of the receptor subunits. We have found that the half life of both major insulin subunits is 9-12 hours. When the cells are cultured in medium containing insulin, the degradation rate is accelerated over 3-fold accounting for the decrease in receptor concentration observed in down-regulation.

Project Description:

In this study we have identified the insulin receptor of the human lymphocyte by biosynthetic labeling with radioactive amino acids (methionine, leucine) and sugars (fucose, galactose, glucosamine and mannose) as well as by external labeling procedures that reveal proteins (lactoperoxidase/$Na^{125}I$) or glycoproteins (galactose oxidase/NaB^3H_4, and periodate/NaB^3H_4). After extraction with Triton X-100, the receptors were precipitated quantitatively and specifically by anti-receptor serum after addition of a second antibody or Protein A. Analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by autoradiography revealed two major bands of molecular weights 135,000 and 95,000 and a minor component of 205,000-210,000. The two major bands were also seen with ^{125}I-insulin covalently cross-linked by disuccinimidyl suberate to lymphocyte plasma membranes.

The interaction of the solubilized insulin receptor from two different human tissues, placenta and cultured lymphocytes with a panel of twelve different lectins has been systematically studied. Six lectins (concanavalin A, lentils, pea, ricin I, ricin II and wheat germ) bound the receptor and resulted in its significant purification. A similar result was obtained with receptors for multiplication stimulating activity (MSA) and epidermal growth factor (EGF). Our findings indicate that the carbohydrate moiety of the insulin receptor contains N-acetyl-glucosamine, mannose, and galactose but that these saccharide residues are neither receptor nor tissue specific.

We have directly demonstrated the glycosylation of the insulin receptor subunits. Receptors of human lymphocytes were biosynthetically labeled with either [^3H]glucosamine, [^3H]galactose, [^3H]fucose, or [^3H]mannose. After solubilization in Triton X-100, cell extracts were immunoprecipitated with serum containing autoantibodies to the insulin receptor. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the immunoprecipitates under reducing conditions showed that both major subunits of the receptor (Mr= 135,000, Mr=95,000) were labeled by all four sugars. The ratio of activity in the 134,000 versus 95,000 Mr bands varied from 2:1 with mannose to 1.2:1 with galactose. In addition, receptor subunits could be demonstrated when the cell surface of intact lymphocytes were labeled with NaB^3H_4 using both the galactose oxidase (acts on non-reducing terminal galactose and N-acetyl-galactosamine) and the periodate technique (oxidizes sialic acid). With the periodate treatment, NaB^3H_4 labeled perferentially the 95,000 Mr band. With the galactose oxidase procedure, on the other hand, NaB^3H_4 labeled only the 135,000 Mr band; prior treatment with neuraminidase increased the labeling of this band and revealed also the 98,000 Mr subunit. These data demonstrate that both major subunits of the insulin receptor are complex glycoproteins with differences in the non-reducing ends of the carbohydrate chains. In the 135,000 Mr subunit, there appears to be more exposed galactosyl and/or N-acetyl-galactosaminyl residues whereas the 95,000 Mr subunit appears to have a higher degree of sialyation.

We have also measured the turnover rate of the polypeptide subunits of the insulin receptor in cultured human lymphocytes and have investigated the mechanism of insulin-induced receptor loss. To estimate the rate of

Project Description:

receptor degradation, lymphocytes were either pulse-labeled with [^{35}S]methionine or surface labeled with Na^{125}I and lactoperoxidase. By both labeling methods, the half lives of the major insulin receptor subunits were 9-12 hours in normal media. When the cells were cultured in media containing 10^{-6}M insulin the turnover was accelerated 2.5 - 3.5 fold ($t_{1/2} \sim 3$ hours). The increase in degradation rate was dependent on the insulin concentration and correlated well with the ability to "down-regulate" the receptor. The rate of biosynthesis of the insulin receptor did not appear to be altered in the presence of 10^{-6}M insulin after correcting for the change in degradation rate. In conclusion, these data demonstrate that insulin-induced receptor loss in cultured lymphocytes is due to accelerated receptor degradation.

Publications:

1. Hedo, J. A., Harrison, L. C., and Roth, J.: Binding of insulin receptors to lectins: Evidence for common carbohydrate determinants on several membrane receptors. Biochem. 20: 3385-3393, 1981.

2. Van Obberghen, E., Kasuga, M., Le Cam, A., Hedo, J. A., Itin, A., and Harrison, L. C.: Biosynthetic labeling of insulin receptor: Studies of subunits in cultured human IM-9 lymphocytes. Proc. Natl. Acad. Sci., USA 78: 1052-1056, 1981.

3. Kasuga, M., Van Obberghen, E., Yamada, K. M., and Harrison, L. C.: Autoantibodies against the insulin receptor recognize the insulin binding subunits of an oligomeric receptor. Diabetes 30: 354-357, 1981.

4. Hedo, J. A., Kasuga, M., Van Obberghen, E., Roth, J., and Kahn, C. R.: Direct demonstration of the glycosylation of insulin receptor subunits by biosynthetic and external labeling: Evidence for heterogeneity. Proc. Natl. Acad. Sci., USA, in press, 1981.

ANNUAL REPORT OF THE PEDIATRIC METABOLISM BRANCH
NATIONAL INSTITUTES OF ARTHRITIS, DIABETES AND DIGESTIVE AND KIDNEY DISEASES

In the fiscal year 1980-1981 clinical and laboratory investigations of cystic fibrosis (CF), a genetic generalized disorder, have continued.

I. GLYCOPROTEIN STUDIES

In cystic fibrosis there is an abnormal physico-chemical behavior of mucous secretions which leads to obstruction of organ passages and consequent chronic obstructive pulmonary disease, pancreatic deficiency, hepatic cirrhosis and various types of intestinal obstruction in neonates and other age groups. Most studies of the chemical structure of glycoproteins have been inconclusive so far, but an intensive effort applying newly available methods has not been done as yet.

Oligosaccharide Structure of Human α_2-Macroglobulin:

Alterations in the carbohydrate moieties of glycoproteins from patients with cystic fibrosis have been described for various glycoproteins. One of these is α_2-macroglobulin which was reported to have decreased sialic acid and altered Concanavalin-A binding when compared to normal α_2-macroglobulin. The possibility that an alteration in the oligosaccharides of glycoproteins is the basic defect in cystic fibrosis is attractive. Changes in the sugar groups of glycoproteins could have consequences ranging from no effect to the elimination of a glycoprotein's biological function. Such a defect could explain the varied experimental results and the assorted clinical symptoms observed in patients with cystic fibrosis.

The primary objective for this study is to elucidate the complete structure of the oligosaccharide chains from normal and cystic fibrosis α_2-macroglobulin. Any differences in the glycoproteins from patients with cystic fibrosis coupled with current knowledge of the synthesis and degradation of oligosaccharide chains on glycoproteins would allow a prediction of where a defect may occur. This could lead to an understanding of the basic defect in cystic fibrosis.

α_2-Macroglobulin was prepared from plasma obtained from single individuals by plasmapheresis. A novel combination of several published procedures was employed to isolate homogenous α_2-macroglobulin. Conventional chemical and enzymatic methods are being used to determine the structure of the oligosaccharide chains. In addition to these conventional techniques, the usefulness of high pressure liguid chromatography to separate and identify oligosaccharides is being investigated. So far, a weak anion exchange column in reverse-phase has provided very good separation of the triantennary chains of fetuin from the biantennary chains of transferrin.

At this point, the analysis of α_2-macroglobulin is ongoing and incomplete and all of the results are preliminary. The data so far accumulated suggests that the α_2-macroglobulin from a normal individual has only one type of oligosaccharide chain, a biantennary, complex chain identical to those of transferrin. The situation may not be as simple for the α_2-macroglobulin in cystic fibrosis since the preliminary methylation analysis is unusual in that it contains much less of 2,4 methylated mannose than does the analysis of the normal sugars. In contrast, the sugar composition of both glycoproteins are very similar if not identical and this analysis fails to confirm a report from another group that cystic fibrosis α_2-macroglobulin is deficient in sialic acid (Mark E. Lowe, Ph.D., NIADDK).

II. THE AUTONOMIC NERVOUS SYSTEM IN CYSTIC FIBROSIS

Altered sensitivity to catecholamines in cystic fibrosis. The fat cells as a model:

Earlier this Branch demonstrated that patients with CF were hyporesponsive to α-adrenergic and cholinergic drugs, but resistant to β-adrenergic drugs as determined by pupillary responses and cardiovascular changes, These findings were consistent with the observation that leukocytes from CF patients produce less cAMP on stimulation in vitro with catecholamines than do cells of controls, We are extending the studies by determination of the sensitivity to catecholamines in a physiologically responsive system, the mature fat cell. The fat cell is an excellent model for such studies as it has been extensively studied and the methodology well standardized. In addition, study of adipose tissue offers the advantage of investigating the responses of tissue obtained directly from patients and carriers of the disease and comparing them with those of subjects not carrying the cystic fibrosis gene.

Adipose tissue obtained by needle biopsy from CF patients, their parents and normal controls is being incubated with isoproterenol and lipolysis determined by increase in glycerol in the medium. In fed, well nourished young men the basal release rate of glycerol from adipose tissue in vitro is negligible.

Isoproterenol (10^{-8} to 10^{-5}M) stimulates lipolysis in confirmation of findings in other laboratories. Through these studies we will determine whether there is a systemic defect in response to catecholamine in the CF patient, what is the nature of the defect and whether the test can be used to detect carriers (heterozygotes) of the CF gene (Milica S. Chernick, M.D., Sidney S. Chernick, Ph.D., NIADDK).

Salivary amylase isozyme:

Some investigators have reported elevated levels of circulating salivary amylase isozyme in patients with CF and feel the observation reflects an autonomic hyperstimulation of the salivary secretion, Our own observations in the past have indicated normal salivary amylase isozyme in sera from CF patients. Studies are ongoing to study a larger group of subjects and directly correlate salivary amylase isozyme activity in serum to levels found directly in salivary gland secretion. During this study, various methodologies for determination of amylase isozyme distribution are being performed in order to ascertain whether some of the apparently discrepant data in the literature can be attributed to methodological differences (Van S. Hubbard, M.D., Ph.D., NIADDK; Robert O. Wolf, D.D.S., NIDR).

III. PLASMA AND CELLULAR AMINO ACID LEVELS IN CYSTIC FIBROSIS.

Alterations in methionine metabolism which have been suggested in cystic fibrosis and the infection and liver involvement present in patients with cystic fibrosis might result in quantitative differences of specific amino acid levels. Therefore, following an overnight fast, blood was obtained from clinically stable cystic fibrosis patients, obligate heterozygotes, and normal subjects. The age range for all subjects was 16-30 years. Plamsa and washed leukocyte pellets were prepared from the blood samples and were processed for quantitative amino acid analysis by standard methods. Cystic fibrosis and normal fibroblasts matched for age and passage number were cultured in three different media (alpha, Eagle's MEM, and MEM + non-essential amino acids), harvested at confluency, and intracellular amino acids measured. Methionine content of all samples derived from cystic fibrosis patients was similar from those derived from normal subjects. Compared to normal plasma (n=6), plasma from cystic fibrosis (CF) patients (n=11) had an increased mean level of glycine (CF 298 nmol/ml ± 12.4 [SEM], normal 231 nmol/ml ± 15.1, $p<.005$) and marginally altered citrulline and phenylalanine; for these amino acids, heterozygote means fell between those of cystic fibrosis homozygotes and normal subjects. The ranges for the concentration of all plasma amino acids overlapped for the three groups studied. When compared to leukocytes derived from normal subjects (n=3), leukocytes from patients with cystic fibrosis (n=5) had reduced leucine (CF 2.54 ± .46, normal 5.00 ± .29), isoleucine (CF 1.01 ± .30, normal 2.51 ± .12), valine (CF 1.52 ± .13, normal 2.94 ± .39), and phenylalanine (CF 0.86 ± .17, normal 1.95 ± .20) expressed as percentage of total amino acids (all $p<0.02$). The ranges for these amino acids within the different groups did not overlap and the amino acid content of leukocytes from obligate heterozygotes (n=4) had decreases of the same magnitude as the homozygote for these four amino acids. The fibroblasts from cystic fibrosis and normal subjects (2 lines each) had similar amino acid content in all three media studied. The alterations noted in the amino acid patterns from cystic fibrosis patients are relatively small, and have no obvious relationship to the major clinical or biochemical features of cystic fibrosis. Since heterozygotes had similar leukocyte amino acid patterns compared to cystic fibrosis homozygotes, it is unlikely that the clinical condition of the patient is responsible for this observation. The number of leukocyte preparations analyzed in this study was small, therefore, further investigations are being performed to determine the reproducibility of this observation and to formulate a mechanism for its occurrence (Van S. Hubbard, M.D., Ph.D., NIADDK; Joseph Schulman, M.D., NICHD).

IV. CLINICAL STUDIES IN CYSTIC FIBROSIS.

These studies are aimed to clarify the significance, incidence, diagnosis and therapy of various abnormalities and complications observed in cystic fibrosis patients of different age groups through the development of clinical studies including the use of experimental diagnostic and therapeutic agents.

1. The use of cimetidine to improve the malabsorption in patients with cystic fibrosis and pancreatic insufficiency. Following observations of reduced steatorrhea and azotorrhea in our initial 6-week clinical trial of using cimetidine as an adjunct to pancreatic enzyme supplementation, we have initiated a long-term clinical trial. The rationale being that cimetidine, an H_2 receptor antagonist, decreases gastric acid output and thereby limits gastric inactivation of the supplemental enzymes. Improvement of gastrointestinal symptomatology has continued as assessed by decreased stool frequency, improved stool consistency, decreased frequency of abdominal discomfort, decreased flatus, and decreased complaints of heartburn. Weight gain has been significant in some patients and no patients have lost weight. Approximately 12 patients have participated in the study, some entering their third year on the medication. No clinical or laboratory side effects have been observed (Van S. Hubbard, M.D., Ph.D., Milica S. Chernick, M.D., Paul A. di Sant'Agnese, M.D., NIADDK).

2. Evaluation of exocrine pancreatic function. A simple method for determination of pancreatic deficiency is very much needed to facilitate genetic and other studies in CF. The evaluation of the synthetic peptide N-benzoyl-L-tyrosyl-p-amino-benzoic acid which is a specific substrate for the proteolytic enzyme,

chymotrypsin, and can be administered orally has been continued. If pancreatic enzymes are present, digestion of this tripeptide occurs and PABA is absorbed, metabolized in the liver and excreted in the urine. Quantitation of aromatic amines in urine collected following the administration of a test dose is then performed. A large number of CF patients with and without pancreatic deficiency as well as other patients with pancreatic deficiency due to other unexplained causes of malabsorption as well as normal volunteers have been studied. Results so far have been in agreement with the other tests used to assess digestion and malabsorption including duodenal drainage, quantitative stool fat, serum amylase isoenzymes, and various lipo-soluble nutrients. These results are encouraging and suggest that this test will be a useful assessment of exocrine pancreatic function. Preliminary studies evaluating recovery of aromatic amines with increasing dosage of supplemental pancreatic enzymes indicate that the test may be useful for titrating enzyme replacement therapy. Improved methods for determination of urinary aromatic amines are being investigated in order to make this test a simple office procedure available to all physicians (Van S. Hubbard, M.D., Ph.D., Milica S. Chernick, M.D., Paul A. di Sant'Agnese, M.D., NIADDK, Robert O. Wolf, D.D.S., NIDR, Robert Fink, M.D., Children's Hospital NMC, Washington, D.C.)

3. Echocardiographic studies in cystic fibrosis. Last year extensive echocardiographic studies in cystic fibrosis were described and an echocardiography score proposed. Longitudinal studies have been continued.

Limited sequential data on 19 patients followed for 1-15 months show that stable patients with initial clinical score of 75-90 had little change in cardiac status (echo score); while patients with moderate disease and scores of 55-65 who deteriorated clinically had increasing abnormal (lower) echo scores. Four of 7 patients with scores of 10 or less (normal echo score = 25) died within 6-12 months of the initial evaluation. Echocardiographic studies of patients started on digitalis because of overt failure showed evidence of significantly improved cardiac status (↑echo score) in 2 of 4 patients. Further longitudinal studies on this patient group and serial studies to monitor various therapeutic interventions are in process (Van S. Hubbard, M.D., Ph.D., NIADDK; Robert Fink. M.D., Children's Hospital NMC, Washington, D.C.; Lucille Lester, M.D., University of Chicago School of Medicine, Chicago, Illinois).

7. Evaluation of skeletal abnormalities in patients with cystic fibrosis. The association of scoliosis with CF is generally not well recognized. To elucidate the matter, we analyzed serial radiographs in 183 cases of CF, looking not only for the presence of spinal deformity, but also seeking evidence of a cause in those affected. The severity and distribution of lung disease was assessed in all patients according to radiological criteria. Pulmonary function tests were also assessed in those patients found to have scoliosis and were correlated with chest radiographs. Clinical and biochemical data were examined for evidence of metabolic bone disease in those affected as opposed to the group as a whole.

A scoliosis of 10% or greater was present in 15 of 151 patients over 4 years of age; 8 males were affected out of 96 and 7 females out of 55 (prevalence 8.3% and 12.7% respedtively); numbers not significantly different than those seen in idiopatic scoliosis. Good correlation was found between the radiological assessment of pulmonary disease and the lung disease as measured clinically and by pulmonary function tests. No evidence of metabolic disease was found either radiologically, biochemically or clinically in any of the 15 cases. The presence of scoliosis was not associated with increased severity of lung disease, nor was there any tendency for those with scoliosis to have predominantly unilateral pulmonary disease (Michael R. Paling, M.D., CC, Milica S. Chermick, M.D., NIADDK).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 49000-08 PMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biological and Physiological Studies of Polyamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David W. Lundgren, Ph.D. Senior Staff Fellow PMB NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

TERMINATED

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 49001-08 PMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Studies in Cystic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Paul A. di Sant'Agnese, M.D. Chief PMB NIADDK

OTHER: Van S. Hubbard, M.D., Ph.D. Senior Surgeon PMB NIADDK
Alan C. Egge, M.D., Surgeon PMB NIADDK
Milica S. Chernick, M.D. Staff Fellow PMB NIADDK
Mark E. Lowe, Ph.D. Staff Fellow PMB NIADDK
Robert Fink, M.D. Guest Worker PMB NIADDK
Vincent McCarthy, M.D. Staff Fellow PMB NIADDK

COOPERATING UNITS (if any)

Lucille A. Lester, M.D. University of Chicago
Sidney S. Chernick, Ph.D. NIADDK
Joseph Schulman, M.D. NICHD

LAB/BRANCH
Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5	PROFESSIONAL: 3	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cystic fibrosis (CF) is a generalized exocrinopathy in which the basic defect is still unknown. Studies have been pursued to determine the characteristics of the disease which are primary and related to a gene defect rather than secondary manifestations. Nutritional assessment of the patients has shown the majority of subjects consume an inadequate number of kilocalories per day to meet their energy requirement and that this observation holds true independent of their degree of pancratic involvement. Methods of improving nutrient intake have included nutritional counselling and a clinical trial using cimetidine.

New diagnostic methods of evaluating the degree of pancreatic insufficiency have been investigated. Ultrasonography has been used to evaluate affected organs with emphasis on cardiac changes which accompany progressive pulmonary disease of CF. These new aids in diagnosing clinical features of CF are especially useful since they offer non-invasive techniques for following longitudinally such functions as cardiac, pancreatic and possibly liver involvement in the CF patient.

Project Description:

Objectives:

These studies are aimed to clarify the significance, incidence, diagnosis and therapy of various abnormalities and complications observed in cystic fibrosis patients of different age groups through the development of clinical studies including the use of experimental diagnostic and therapeutic agents.

1. The use of cimetidine to improve the malabsorption in patients with cystic fibrosis and pancreatic insufficiency. Following observations of reduced steatorrhea and azotorrhea in our initial 6-week clinical trial of using cimetidine as an adjunct to pancreatic enzyme supplementation, we have initiated a long-term clinical trial. The rationale being that cimetidine, an H_2-receptor antagonist, decreases gastric acid output and thereby limits gastric inactivation of the supplemental enzymes. Improvement of gastrointestinal symptomotology has continued as assessed by decreased stool frequency, improved stool consistency, decreased frequency of abdominal discomfort, decreased flatus, and decreased complaints of heartburn. Weight gain has been significant in some patients and no patients have lost weight. Approximately 12 patients have participated in the study, some entering their third year on the medication. No clinical or laboratory side effects have been observed.

2. Evaluation of exocrine pancreatic function. A simple method for determination of pancreatic deficiency is very much needed to facilitate genetic and other studies in CF. The evaluation of the synthetic peptide N-benzoyl-L-tyrosyl -p-amino-benzoic acid which is a specific substrate for the proteolytic enzyme, chymotrypsin, and can be administered orally has been continued. If pancreatic enzymes are present, digestion of this tripeptide occurs and PABA is absorbed, metabolized in the liver and excreted in the urine. Quantitation of aromatic amines in urine collected following the administraiton of a test dose is then performed. A large number of CF patients with and without pancreatic deficiency as well as other patients with pancreatic deficiency due to other unexplained causes of malabsorption as well as normal volunteers have been studied. Results so far have been in agreement with the other tests used to assess digestion and malabsorption including duodenal drainage, quantitative stool fat, serum amylase isoenzymes, and various lipo-soluble nutrients. These results are encouraging and suggest that this test will be a useful assessment of exocrine pancreatic function. Preliminary studies evaluating recovery of aromatic amines with increasing dosage of supplemental pancreatic enzymes indicate that the test may be useful for titrating enzyme replacement therapy. Improved methods for determination of urinary aromatic amines are being investigated in order to make this test a simple office procedure available to all physicians.

3. Echocardiographic assessment of cardiac performance and development of a new echocardiographic scoring system in cystic fibrosis (CF). To characterize the sequence of cardiac changes that accompany the progressive pulmonary disease of CF, we performed M-mode echocardiography. Echocardiographic measurements of right and left heart dimensions and left and right ventricular systolic time intervals have been compared to data previously obtained from normals. At the time of the echocardiograms all children had a complete evaluation of their pulmonary

status which included a chest roentgenogram and EKG; pulmonary function testing (if age 6 or more), and clinical scoring.

The right ventricular wall thickness was increased in 70% of patients, and the right ventricular dimension was increased in 42% of patients, 90% of whom also had increased wall thickness. Increasing right ventricular dilatation was variably associated with alterations of left heart size and function. When echo abnormalities were examined in relation to Shwachman Clinical Score we found that the right ventricular wall thickness was increased in 35% of patients with mild disease (Clinical score (CS) = 81-100), 72% of moderate (CS = 61-80), and in 100% of patients characterized as having moderately-severe (CS = 45-60) or severe (CS = F45) disease involvement. The right ventricular dimension was increased in 15% of mild; 28% of moderate, 83% of moderately-severe and 100% of patients with severe disease. RV systolic time intervals were abnormal in 50% of patients with moderately severe and 100% of patients with severe disease, and LV systolic time intervals were abnormal in the more severely affected patients in particular those with overt right heart failure.

Based on an apparent sequence of echo abnormalities in the progression from mild to severe disease an echo scoring system has been developed. This scoring system which provides an objective, quantitative method for characterizing the cardiac changes that occur in this progressive pulmonary disease correlated very well with Shwachman (r=0.87) and Taussig NIH (r=0.89) clinical scores, chest x-ray scores (r=0.86) and pulmonary function test results in these patients.

Limited sequential data on 19 patients followed for 1-15 months show that stable patients with initial clinical score of 75-90 had little change in cardiac status (echo score); while patients with moderate disease and scores of 55-65 who deteriorated clinically had increasing abnormal (lower) echo scores. Four of 7 patients with scores of 10 or less (normal echo score = 25) died within 6-12 months of the initial evaluation. Echocardiographic studies of patients started on digitalis because of overt failure showed evidence of significantly improved cardiac status (↑echo score) in 2 of 4 patients. Further longitudinal studies on this patient group and serial studies to monitor various therapeutic interventions are in process.

4. Plasma and cellular amino acid levels in cystic fibrosis. Alterations in methionine metabolism which have been suggested in cystic fibrosis and the infection and liver involvement present in patients with cystic fibrosis might result in quantitative differences of specific amino acid levels. Therefore, following an overnight fast, blood was obtained from clinically stable cystic fibrosis patients, obligate heterozygotes, and normal subjects. The age range for all subjects was 16-30 years. Plasma and washed leukocyte pellets were prepared from the blood samples and were processed for quantitative amino acid analysis by standard methods. Cystic fibrosis and normal fibroblasts matched for age and passage number were cultured in three different media (alpha, Eagle's MEM, and MEM + non-essential amino acids), harvested at confluency, and intracellular amino acids measured. Methionine content of all samples derived from cystic fibrosis patients was similar from those derived from normal subjects. Compared to normal plasma (n=6), plasma from cystic fibrosis (CF) patients (n=11) had an increased mean level of glycine (CF 298 nmol/ml ± 12.4 [SEM], normal 231 nmol/ml ± 15.1, $p<.005$) and marginally altered citrulline and phenylalanine; for

these amino acids, heterozygote means fell between those of cystic fibrosis homozygotes and normal subjects. The ranges for the concentration of all plasma amino acids overlapped for the three groups studied. When compared to leukocytes derived from normal subjects (n=3), leukocytes from patients with cystic fibrosis (n=5) had reduced leucine (CF 2.54 ± .46, normal 5.00 ± .29), isoleucine (CF 1.01 ± .30, normal 2.51 ± .12), valine (CF 1.52 ± .13, normal 2.94 ± .39), and phenylalanine (CF 0.86 ± .17, normal 1.95 ± .20) expressed as percentage of total amino acids (all $p<0.02$). The ranges for these amino acids within the different groups did not overlap and the amino acid content of leukocytes from obligate heterozygotes (n=4) had decreases of the same magnitude as the homozygote for these four amino acids. The fibroblasts from cystic fibrosis and normal subjects (2 lines each) had similar amino acid content in all three media studied. The alterations noted in the amino acid patterns from cystic fibrosis patients are relatively small, and have no obvious relationship to the major clinical or biochemical features of cystic fibrosis. Since heterozygotes had similar leukocyte amino acid patterns compared to cystic fibrosis homozygotes, it is unlikely that the clinical condition of the patient is responsible for this observation. The number of leukocyte preparations analyzed in this study was small, therefore, further investigations are being performed to determine the reproducibility of this observation and to formulate a mechanism for its occurrence.

5. Autonomic nervous system dysfunction in patients with cystic fibrosis. Investigations of abnormalities relating to the autonomic nervous system have continued. Some investigators have reported elevated levels of circulating salivary amylase isozyme in patients with CF and feel the observation reflects an autonomic hyperstimulation of the salivary secretion. Our own observations in the past have indicated normal salivary amylase isozyme in sera from CF patients. Studies are ongoing to study a larger group of subjects and directly correlate salivary amylase isozyme activity in serum to levels found directly in salivary gland secretion. During this study, various methodologies for determination of amylase isozyme distribution are being performed in order to ascertain whether some of the apparently discrepant data in the literature can be attributed to methodological differences.

6. Altered sensitivity to catecholamines in cystic fibrosis. Earlier this Branch demonstrated that patients with CF were hyporesponsive to α-adrenergic and cholinergic drugs, but resistant to β-adrenergic drugs as determined by pupillary responses and cardiovascular changes. These findings were consistent with the observation that leukocytes from CF patients produce less CAMP on stimulation in vitro with catecholamines than do cells of controls. We are extending the studies by determination of the sensitivity to catecholamines in a physiologically responsive system, the mature fat cell.

Adipose tissue obtained by needle biopsy from CF patients, their parents and normal controls are being incubated with isoproterenol and lipolysis determined by increase in glycerol in the medium. In fed, well nourished young men the basal release rate of glycerol from adipose tissue in vitro is negligible.

Isoproterenol (10^{-8} to 10^{-5}M) stimulates lipolysis in confirmation of findings in other laboratories. Through these studies we will determine whether there is a systemic defect in response to catecholamine in the CF patient, what is the nature of the defect and whether the test can be used to detect carriers (heterozygotes) of the CF gene.

7. Evaluation of skeletal abnormalities in patients with cystic fibrosis. The association of scoliosis with CF is generally not well recognized. To elucidate the matter, we analyzed serial radiographs in 183 cases of CF, looking not only for the presence of spinal deformity, but also seeking evidence of a cause in those affected. The severity and distribution of lung disease was assessed in all patients accordably to radiological criteria. Pulmonary function tests were also assessed in those patients found to have scoliosis and were correlated with chest radiographs. Clinical and biochemical data were examined for evidence of metabolic bone disease in those affected as opposed to the group as a whole.

A scoliosis of 10% or greater was present in 15 of 151 patients over 4 years of age; 8 males were affected out of 96 and 7 females out of 55 (prevalence 8.3% and 12.7% respectively); numbers not significantly different than those seen in idopatic scoliosis. Good correlation was found between the radiological assessment of pulmonary disease and the lung disease as measured clinically and by pulmonary function tests. No evidence of metabolic disease was found either radiologically, biochemically or clinically in any of the 15 cases. The presence of scoliosis was not associated with increased severity of lung disease, nor was there any tendency for those with scoliosis to have predominantly unilateral pulmonary disease.

Significance to Biomedical Research:
These studies will aid in the investigations of the basic hereditary defect in CF, the most frequent semi-lethal genetic disease in this country, by defining and improving the diagnostic and therapeutic approach to the many manifestations and/or complications of patients with CF of various ages. The approach to counseling about their disease and risk of complications may vary dependent on their age, degree of pancreatic involvement, and development of secondary complications. Additionally, careful studies comparing CF patients with and without clinical exocrine pancreatic insufficiency will allow us to understand more about the role of nutrition in cystic fibrosis and interpret our results more intelligently and thereby possibly come closer to the identification of the basic defect.

Publications:

Hubbard, V.S., Dunn, G.D. and Lester, L.A.: Effectiveness of cimetidine as an adjunct to supplemental pancreatic enzymes in patients with cystic fibrosis. Am. J. Clin. Nutr. 33:2281-2286, 1980.

Davis, P.B., Hubbard, V.S. and di Sant'Agnese, P.A.: Low sweat electrolytes in cystic fibrosis. Am. J. Med. 69:643-646, 1980.

Lester, L.A., Egge, A.C., Hubbard, V.S., Camerini-Otero, C.S. and Fink, R.J.: Echocardiography in cystic fibrosis: A proposed scoring system. J. Pediatr. 97:742-748, 1980.

Wolf, R.O. and Hubbard, V.S.: Serum salivary and pancreatic amylase. Pediatr. Res. 14:968, 1980.

Davis, P.B. and Laudon, S.C.: Adenylate cyclase in leukocytes from patients with cystic fibrosis. J. Lab. and Clin. Med. 96:75-84, 1980.

Spicer, S.S., di Sant'Agnese, P.A., Vincent, R.A. and Ulane, M.: Ultrastructural and cytochemical comparison of cultured normal and cystic fibrosis fibroblasts. Experimental and Molecular Pathology. 33:104-121, 1980.

Cohen, L.F., di Sant'Agnese, P.A. and Friedlander, J.: Cystic fibrosis and pregnancy: A national survey. Lancet 2:842-844, 1980.

di Sant'Agnese, P.A., Hubbard, V.S. and Lowe, M.E.: Recent developments in clinical and basic research in cystic fibrosis. Monographs in Paediatrics, Karger, 1981, (in press).

Bieri, J.G., Corash, L. and Hubbard, V.S.: Medical progress: Medical uses of vitamin E. N. Engl. J. Med. (in press).

Hubbard, V.S. and Mangrum, P.: Energy intake and nutritional counseling in cystic fibrosis. J. Am. Diet Assoc. (in press).

Paling, M.R. and Spasovska-Chernick, M.: Scoliosis in cystic fibrosis: An appraisal. Skeletal Radiology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 49004-02 PMB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Fatty Acids and Membrane Function in Cystic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Van S. Hubbard, M.D., Ph.D. Senior Surgeon PMB NIADDK

COOPERATING UNITS (if any)

John Bieri, Ph.D. LNE NIADDK
Mary C. McKenna, Ph.D. LNE NIADDK

LAB/BRANCH

Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The fatty acid composition of blood and tissue lipids of patients with cystic fibrosis has been shown to be abnormal. The predominant alterations include a decreased linoleic acid and increased palmitoleic and oleic acids. These alterations are primarily due to the malabsorption and maldigestion associated with pancreatic insufficiency in the cystic fibrosis patient and are not corrected by exogenous pancreatic enzyme supplementation. The major unsaturated fatty acid within phospholipids, and thus within membrane lipids is linoleic acid. Decreased linoleic acid content as observed in most cystic fibrosis patients is thus associated with decreased unsaturation in tissue phospholipids and membranes. This characteristic may be related to alterations in membrane function as well as altering prostaglandin synthesis. Current work is aimed at delineating the role of altered fatty acid composition of blood and membrane lipids as a cause of alterations in the recognition or interaction of the beta-receptors in CF tissues and alteration in the availability of arachidonic acid for the biosynthesis of prostaglandin.

Project Description:

Objectives:

The fatty acid composition of plasma and tissue lipids of patients with cystic fibrosis has been shown to be abnormal. Our work during the past several years has documented that this generalized observation is a result of the malabsorption and maldigestion associated with pancreatic insufficiency in the cystic fibrosis patient and cannot be corrected by any current routine therapeutic intervention. Since 85-90% of the cystic fibrosis patients are observed to have this abnormality of fatty acid composition, it is important to determine how this altered fatty acid composition influences both in vitro and in vivo studies from patients with cystic fibrosis.

Fatty acid composition of blood and tissue lipids in cystic fibrosis:

We have previously demonstrated that the abnormal fatty acid composition of plasma lipids observed in patients with CF is a secondary effect of the associated pancreatic insufficiency (Hubbard et al, Lancet 2:1302-1304, 1977). This observation has subsequently been confirmed by two other groups (Galabert et al, Lancet 2:903, 1978, Mishler et al, CF Club Abstracts, 1979). We have documented (Clinica Chemica Acta 102:115-118, 1980) that the abnormality of the fatty acid composition of erythrocyte phospholipids in CF patients is also only observed in the presence of pancreatic insufficiency. However, several other groups are still conducting studies relating the defect in fatty acid metabolism to the etiology of CF. It is possible that if the fatty acid composition of specific phospholipids (i.e.: phosphotidylethanolamine, phosphotidylserine and phosphatidylcholine) were determined smaller differences might be recognized.

In a small number of monocyte preparations increased percentage of arachidonic acid in the total phospholipid fraction was observed in samples derived from CF patients compared to normal subjects. This observation as well as the decreased responsiveness of leukocytes to stimulation by isoproterenol reported by Davis from our lab in the past can be explained by observations of Hirata et al (PNAS 75:1718, 1978, PNAS 76:368, 1979). This group has shown that in the erythrocyte, reticulocyte and other cells, there is a transmethylation pathway present in plasma membrane which is catalyzed by two sepatate enzymes. Their investigations have shown that if there is a defect in this pathway, an uncoupling phenomenon occurs between the stimulation of the beta receptor and the formation of cyclic AMP. Additionally, if there is a defect in the transmethylation pathway, one would predict that the concentration of phosphotidylethanolamine and phosphotidylserine on the inner side of the plasma membrane would be increased along with a decrease in the biosynthesis and subsequent concentration of phosphotidylcholine on the outer side of the membrane. These events would account for an increase in the arachidonic acid in phospholipids derived from such cells since arachidonic acid is the predominant fatty acid in the C-2 position of phosphotidylserine and phosphotidylethanolamine and in the normal situation the fatty acid in the C-2 position of phosphotidylcholine on the outer side of the membrane is cleaved by phospholipase A-2 to yield lysolecithin. Preliminary studies in patients both with and without pancreatic insufficiency indicate that there may be a defect in this transmethylation pathway of phospholipids when using an enzyme preparation derived from the leukocytes of these patients. Problems with obtaining a purified leukocyte plasma membrane preparation has hindered extension of these studies. Other cell types are currently being investigated to determine if the hyporesponsiveness to isoproterenol is present; if observed, these cell types (i.e.: fat cell) will be used to extend the above studies.

Recent studies have suggested that CF patients without malabsorption have a slower progression of their disease. It is not known whether this occurs because of their nutritional status (including normal fatty acid composition) or because they may have a slightly different genetic disease. Since patients with normal and abnormal absorption occur in the same family and those with normal absorption are observed to eventually lose pancreatic function, nutritional factors may be the predominant explanation for some of the morbidity of the disease.

To investigate the role of altered fatty acid composition, (with emphasis on levels of the essential fatty acid, linoleic acid), specific forms of dietary supplements are being evaluated to assess absorption in and acceptance by the CF patient with pancreatic insufficiency. As efficacious forms of linoleic acid supplements are established, assessment of supplementation programs will be performed including monitoring pulmonary function studies, hemoglobin-oxygen dissociation curves, 2,3-DPG levels, clinical score, activity level, and number of hospitalizations.

Significance to Biomedical Research:

Because the altered fatty acid composition of tissue lipids may affect their function, routine assessment of the fatty acid status of our patients when diagnosed and at periodic intervals is obtained. Determinations at time of diagnosis are useful in characterizing the patient, especially in regard to the clinical evaluation of exocrine pancreatic function. The fatty acid composition, especially with regard to the degree of unsaturation may account for some of the differences observed in other CF research and may be a partial explanation for the variability seen from patient to patient. Thus, this analysis is used as an aid in interpreting other research data obtained from our patient population.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 49005-02 PMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Follow-up of Hypochloremic Infant Formula Feedings

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Van S. Hubbard, M.D., Ph.D. Senior Surgeon PMB NIADDK

COOPERATING UNITS (if any)
Artemis Simopoulos, M.D. OD
Charles Chang, M.D. DMNB NINCDS
Berry Bercu, M.D. NPMB NICHD
Howard Moss, Ph.D. LDP NIMH

LAB/BRANCH
Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three cases of Bartter-like syndrome in infants were reported to the Center for Disease Control on July 26, 1979. These infants were less than 10 months of age, failed to gain weight, had poor appetites, and were observed to have hypochloremia, hypokalemia, and varying degrees of alkalosis. Subsequently approximately 141 infants have been documented to have experienced the same constellation of symptoms. The common denominator in all these cases is thought to be the consumption of the same brand of soy-base formula, Neo-Mull-Soy. It was learned that this formula contained deficient amounts of chloride and infants using this formula as the sole or predominant source of food developed a severe chloride deficiency with other associated metabolic alterations. Long-term effects of chloride-deficient feedings in humans is not known and therefore a follow-up of these infants has been initiated.

Project Description:

Objectives:

Investigations of the possible long-term complications of chloride deficient formula feedings and metabolic abnormalities associated with these infant formulas and of the possibility of certain infants within this group having a pre-disposition to the development of metabolic abnormalities during a low chloride intake are being undertaken.

Methods:

A five-year follow-up study of infants who were fed one of the two soy-bean based formulas deficient in chloride, an essential nutrient, has been initiated. Chloride deficiency can cause metabolic alkalosis which has been associated with a failure to thrive or a delay in the growth and development of affected infants. In July, 1979 a pediatric nephrologist reported to the Food and Drug Administration and to the Center for Disease Control 3 cases of metabolic alkalosis in infants who were being fed only Neo-Mull-Soy, one of the soy formulas. Further investigations have found at least 141 cases of infants having episodes of metabolic alkalosis (many having a blood pH of 7.60 or greater) associated with feedings of Neo-Mull-Soy or CHO-Free, the other formula. Analysis of formula preparations has shown that the products contained one-third of the chloride stated on the product label and supplied approximately one-fifth of the chloride recommended for infants by the American Academy of Pediatrics.

Studies have been selected in order to rule out other common causes of metabolic alkalosis especially Bartter's syndrome which has symptoms similar to those observed in these infants. These studies have included clinical evaluation with emphasis of growth and development, and detailed neurological and psychological examination. Laboratory evaluation of the metabolic status of the infants has also been monitored. Twenty-three infants have been entered into the study as of this time. Although all infants have had documented metabolic alkalosis in association with Neo-Mull-Soy consumption, symptomatic expression of the disorder has been variable. Distinct sequelae relating to the deficient chloride feeding has not yet been established.

Significance to Biomedical Research:

Since the occurence of the chloride deficient feedings in these infants has been likened to an "experiment in nature", it offers a unique opportunity to study the important role of chloride in growth and development in humans. The time of the chloride deficiency in relationship to the rapid rate of growth which occurs in the child at such a time makes further understanding of all nutrient requirements of utmost importance.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL** RESEARCH PROJECT	PROJECT NUMBER Z01 AM 49006-01 PMB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Oligosaccharide Structures of Human α_2-Macroglobulin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Mark E. Lowe, Ph.D. PMB NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

α_2-Macroglobulin was isolated from the plasma of normal individuals and patients with cystic fibrosis. An analysis of the carbohydrate structures on these homogenous preparations is ongoing. The preliminary results indicate that the α_2-macroglobulin from normal and cystic fibrosis patients have similar sugar compositions, but that the oligosaccharide chains may have different sequences or linkages.

Project Description:

Objectives:

The primary objective for this study is to elucidate the complete structures of the oligosaccharide chains from normal and cystic fibrosis α_2-macroglobulin. Any differences in the glycoproteins from patients with cystic fibrosis coupled with current knowledge of the synthesis and degradation of oligosaccharide chains on glycoproteins would allow a prediction of where a defect may occur. This could lead to an understanding of the basic defect in cystic fibrosis.

Methods:

Isolation of α_2-macroglobulin: α_2-macroglobulin was prepared from plasma obtained from single individuals by plasmapheresis. A novel combination of several published procedures was employed to isolate homogenous α_2-macroglobulin. The plasma was first precipitated with polyethylene glycol. Then, the concentrated sample was passed over a column of Blue-dextran sepharose-6B, followed by zinc chelate chromatography. Finally, the sample was chromatographed on Sephacryl S-300. About 300 to 400 mg. of α_2-macroglobulin was prepared from 300 mls of plasma.

Carbohydrate Analysis: Conventional chemical and enzymatic methods are being used to determine the structure of the oligosaccharide chains. The alditol acetates of the sugars are examined by gas liquid chromatography and gas chromatography-mass spectrometry to establish the sugar compositions. The sugar linkages are elucidated by methylation analysis. The order of the carbohydrates is determined by sequential treatment with specific glycosidases and by Smith degradations. The intact glycoprotein, glycopeptides prepared by exhaustive pronase digestion, and oligosaccharides released by trifluoroacetolysis are all used as the starting material for the various procedures.

In addition to these conventional techniques, the usefulness of high pressure liquid chromatography to separate and identify oligosaccharides is being investigated. So far, a weak anion exchange column in reverse-phase has provided very good separation of the triantennary chains of fetuin from the biantennary chains of transferrin.

Results:

At this point, the analysis of α_2-macroglobulin is ongoing and incomplete and all of the results are preliminary. The data so far accumulated suggests that the α_2-macroglobulin from a normal individual has only one type of oligosaccharide chain, a biantennary, complex chain identical to those of transferrin. The situation may not be as simple for the α_2-macroglobulin in cystic fibrosis since the preliminary methylation analysis is unusual in that it contains much less of 2,4 methylated mannose than does the analysis of the sugars from normal α_2-macroglobulin. In contrast, the sugar composition of both glycoproteins are very similar if not identical and this analysis fails to confirm a report from another group that cystic fibrosis α_2-macroglobulin is deficient in sialic acid.

Significance to Biomedical Research:

Alterations in the carbohydrate moieties of glycoproteins from patients with cystic fibrosis have been described for various glycoproteins. One of these is α_2-macroglobulin which was reported to have decreased sialic acid and altered Concanavalin-A binding when compared to normal α_2-macroglobulin. The possibility that an alteration in the oligosaccharides of glycoproteins is the basic defect in cystic fibrosis is attractive. Changes in the sugar groups of glycoproteins could have consequences ranging from no effect to the elimination of a glycoprotein's biological function. Such a defect could explain the varied experimental results and the assorted clinical symptoms observed in patients with cystic fibrosis.

Publications:

None.

ANNUAL REPORT SUMMARY

CLINICAL HEMATOLOGY BRANCH

I. Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis.

A. Enhancement of Platelet Function by Inhibition of Phospholipid Methylation

Phospholipid methylation studied in a variety of mammalian cells has been linked to a number of events occurring following stimulation by receptor-agonist association. Processes that initiate cellular response such as activation of adenylate cyclase or phospholipase, changes in membrane fluidity and ionic flux, all have been reported to be dependent on methylation of plasma membrane phospholipids. Inhibition of methylation had been found to suppress physiologic responses of basophiles, neutrophils, fibroblasts, lymphocytes and some mammalian established cell lines to specific stimuli. We had previously observed that inhibition of phospholipid methylation in platelets through the competitive activity S-adenosyl-homocystine and 3-deazaadenosine, did not suppress platelet responses to usual concentrations of the agonists, thrombin, ADP, epinephrine, collagen, arachidonic acid or A23187 as measured by serotonin release, platelet aggregation, malondialdehyde formation, clot traction or arachidonic acid release. These observations suggested that, in contrast to all other cells studied, platelet function was not decreased by inhibitors of phospholipid methylation. Further studies of platelet function showed that methyltransferase inhibitors at levels which completely suppress phospholipid methylation actually sensitized the platelet response, in response to minimum stimulatory concentrations of agonists. Whether perturbation of the methylation pathway directly increases the sensitivity of platelet response to various agonists, or whether the agents used to block methylation increased platelet response through, as yet, unidentified metabolic affects is currently under study.

B. Effects of Platelet-inhibitory Drugs on Coronary and Cardiac Collateral Blood Flow Relative to Their Effects on Platelet Function.

Several drugs known to inhibit platelet function appear to have value in preventing recurrence of coronary thrombosis or coronary insufficiency. The effectiveness of these drugs in the treatment of heart disease has been assumed to be related to inhibition of platelet function, possibly to prevention of platelet thrombus formation that may lead to coronary artery occlusion. the alternate possibility that these drugs may have a direct affect on the cardiac vasculature has not been assessed. In conjunction with investigators in the Heart Institute we have compared the effects of sulfinpyrazone, ibuprofen, indomethacin, aspirin, and verapamil, on platelet function and coronary and collateral cardiac blood flow to determin whether therapeutic benefits ascribed to these various drugs could be attributed primarily to their affects on platelets or on myocardial vasculature. By comparing circulator responses *in vivo* with *in vitro* function over the entire dose-response range of the various drugs, it was found that selfinpyrazone and aspirin increased epicardial coronary collateral flow in the same dosage range that inhibited platelet aggregation responses to various agonists. Thus enhanced collateral flow may be responsible for the beneficial affects ascribed to these drugs in coronary artery disease, and inhibition of platelet function may be of secondary importance. In a case of verapamil, the usual doses producing an apparent beneficial effect in vivo had no affect on platelet function in vitro. However, at in vitro concentrations of verapamil higher than those attained in vivo, platelet function was inhibited. The responsiveness of platelets from various normal donors to verapamil varied widely, and this variation appeared to be correlated with a similar variation in cardiac responsiveness to verapamil at lower doses. These observations suggest that the sensitivity of the platelet-inhibitory response to verapamil may serve as an index of the cardiac responsiveness of different individuals to this drug. Studies of effects of indomethacin and ibuprofen indicate that platelet-inhibitory doses of these drugs are similar to the doses that interfere with prostaglandin-mediated coronary vasodilation. The latter drugs, by inhibiting cyclooxygenase, equally suppress prostacyclin-induced vasodilation and thromboxane-induced platelet aggregation. The net affect on coronary vascular events would not be readily predictable.

C. The Effects of Anion Transport-Blocking Agents and Alterations of Osmotic Strength on the Interaction Between Platelets and Thrombin.

The ability of anion transport blocking agents and of elevated osmotic strength to inhibit exocytotic processes following stimulation of various cells or chromaffin granules by a variety of secretory agents has led to the concept that the mechanism of exocytosis is a chemiosmotic process. Platelet secretion is suppressed by anion transport blocking agents and elevated osmotic strength in all respects similar to that of other secretory cells that have been studied. The chemiosmotic theory suggests that the biochemical events in cellular secretion proceed to the point at which secretory granule fusion with plasma membrane occurs, then ionic transport and osmotic rupture accounts for release of granular material in the final stage of exocytosis. Anion transport inhibition and high osmotic strength supposedly inhibit this last stage of exocytosis. We found in our previous studies concerning the metabolic effects of anion transport blockers and high osmotic strength on platelets that the biochemical responses to stimuli were inhibited proportionately to inhibition of the platelet secretion. Moreover, anion transport blockers and high osmotic strength suppressed malondialdehyde formation which is the earliest metabolic response of platelets to stimulation. Furthermore, although anion transport blockers and high osmotic strength inhibited platelet release caused by thrombin, these inhibitors had no effect on release initiated by other stimuli such as ADP or A23187. These various observations are inconsistent with concepts of chemiosmotic regulation by anion transport blockers and high ionic strength. Because the platelet receptor for thrombin may be platelet-associated fibrinogen we studied the effect of anion transport blockers and high osmotic strength on the conversion of fibrinogen to fibrin by thrombin. This reaction was inhibited to the degree comparable to the degree of inhibition of platelet secretion by similar concentrations of anion transport blockers and levels of increased ionic strength. We next determined whether inhibition of platelet secretion was a result primarily of suppressed decreased binding of thrombin. It is clear that the effects of anion transport blockers and increased osmotic strength on platelet function do not provide evidence for a chemiosmotic step in exocytosis as previously supposed. Other cells that have been studied from this point of view should be re-evaluated to determine whether interference with agonist attachment to receptor accounts for the action of anion channel blockers and increased osmotic strength rather than interference with a chemiosmotic lytic process.

II. The Study of Immunology of Blood Cell Deficiencies

A. Evaluation of Highly Sensitive Immunoglobulin Assays for Detection of Anti-Platelet Antibodies of Clinical Significance

Many of the isoantibodies that arise clinically as a result of platelet transfusions or fetal-maternal immunization cannot be detected by conventional serologic tests involving cellular agglutination or complement fixation, either because the antibodido not possess these inherent properties or because antibody affinity or concentration is too low to support these reactions. Tests based on the use of labelled antiglobulin reagents or stapholococcal proten A have been considered the most sensitive for detecting minimal concentrations of cell-associated immunoglobulin. Using a variety of known isoantibodies standardized in our laboratory on the basis of complement fixation, cellular agglutination, serotonin release and platelet aggregation we compared the sensitivity of labelled antiglobulin and stapholococcal protein A assays with that of conventional tests. Antiglobulin and protein A reactions were no more sensitive than complement fixation tests with potent complement-fixing antibodies but were more suitable for detecting some isoantibodies that did not have complement-fixing properties. However, all of the tests were from 10 to 100-fold less sensitive than the requirement for detecting in vivo concentrations of antibody that caused thrombocytopenia. By in vivo tests we had found that 200 to 800 antibody molecules attached per cell can cause thrombocytopenia whereas, the most sensitive in vitro immunologic tests detected no less than 2,000 to 5,000 antibody molecules per cell. One of the major reasons for the relative insensitivity of in vitro tests is the necessity for washing cells exposed to antiglobulin or staph protein A reagents. This results in extensive elution of the relatively low affinity antibodies that commonly form clinically. Current plans concern development of modified antiglobulin and protein A reactions involving exposure of purified platelet membrane components to antibody in systems that can be controlled without washing procedures.

B. Platelet-Associated IgG as a Measure of Mean Platelet Age and Platelet Turnover

In the past several years there have been numerous reports on elevated platelet-associated IgG (PAIgG) in patients with idiopathic thrombocytopenic purpura (ITP). Because the platelet life span is decreased in this disease and there is a factorin the plasma of patients with ITP (ITP Factor) that decreases the platelet level when transfused into normal recipients, ITP has been considered to be an antibody-mediated disorder. The finding of elevated PAIgG has been taken as further proof of this etiology and evaluation of PAIgG has been suggested as a diagnostic, as well as prognostic test. We have corroborated the finding that PAIgG is elevated in ITP but we disagree with interpretation of these results. The amount of PAIgG found on platelets in our studies exceeded the saturating amount of known isoantibodies and drug antibodies by 1 to 2 orders of magnitude and exceeded the minimum injurious concentration of known antibodies by as much as 3 to 4 orders of magnitude. Since most patients with ITP having these high concentrations of PAIgG did not have severe depression of platelets, it appeared unlikely that the immunoglobulin coating the platelets was true antibody. In addition, when patients' platelet levels returned to normal after splenectomy or steroid treatment the PAIgG could return to normal within days, a finding inconsistent with the known persistence of ITP factor in the plasma of patients who had responded to these forms of therapy. The persistence of ITP factor has been measured in our laboratory by passive transfer experiments. Moreover, it appears that some patients with thrombocytopenia due to destruction of platelets by non-immunologic mechanisms also had high levels of PAIgG. These various considerations indicate that PAIgG is not a measure of attachment of true antibodies to platelets, but rather is an indication of exaggerated absorption of non-antibody IgG to immature platelets. When platelets are turning over more rapidly, as they do in ITP, the average age of platelets is markedly reduced. Since platelets normally contain on the order of 4,000 to 6,000 molecules of IgG per platelet and the membranes of younger platelets tend to be more highly absorptive, the test for PAIgG may prove to be an indication of platelet mean age that is, an index of the rate of platelet turnover rather than an indication of coating by antibodies. We are currently pursuing experiments to further define the significance of PAIgG.

C. Serologic, Clinical Course and Therapy of Isoimmune Neonatal Thrombocytopenia (INT)

INT occurs in otherwise normal children as a result of transplacental transfer of maternal antibody stimulated by incompatible fetal platelet antigens. The frequency is about one in 5,000 births and there is a mortality of approximately 13% in untreated cases. We have recently analyzed our results of testing serum and platelets obtained on 224 normal mothers whose newborn infants were suspected of having INT, and have evaluated the outcome of various forms of treatment in 44 families with 2 or more children in which INT occurred. The major findings of this study were that approximately 50% of all cases of this disorder were caused by sensitization to the Pl^{A1} antigen which we initially defined 20 years ago. All of the antibodies elicited by this particular antigen are non-complement fixing and non-agglutinating and are present at low concentrations, rarely detectable by conventional serologic tests. We have defined the frequency of occurrence of INT as a result of sensitization to 6 other platelet antigens and established diagnostic criteria for these cases. In this largest series of cases available to-date in addition to 55 cases studied up to 1964, we found that the most effective form of therapy is platelet transfusion with washed maternal platelets, that exchange transfusion is of limited value unless combined with platelet transfusion, and that adrenocorticosteroid therapy is relatively ineffective. We established serologic criteria for predicting the likelihood of recurrence of INT in subsequent children after an index case. This will permit planning for therapeutic procedures in future cases and further evaluation of the effectiveness of caesarean section in preventing hemorrhagic complications due to birth trauma. We established that maternal fetal ABO incompatibility is not a susceptibility factor in INT despite some information in the literature suggesting this possibility.

D. Serology, Clinical Course and Therapy of Post-transfusion Purpura (PTP)

The syndrome of PTP which we defined 20 years ago is characterized by the sudden onset of thrombocytopenia approximately a week following transfusion with blood or blood fractions containing platelet material. When thrombocytopenia develops there is an antibody in the patient's serum against an isoantigen present on transfused platelets but absent on the recipient's platelets. This unusual syndrome, relevant to disorders of autoimmunity, has been studied by us in 34 additional cases. This year we reviewed the serology, clinical course, and therapy of these cases, as well as 14 other reported patients. Of special interest are the findings that all cases appear to be due to a single antigen incompatibility, Pl^{A1}, that the reaction occurs only after an anamnestic antibody response, and that the antibody apparently in association with residual circulating platelet antigen is adsorbed by the patients' platelets to cause thrombocytopenia. This mechanism of platelet destruction fits the findings that recovery takes place in the face of persistent antibody and that the amount of platelet antigen received during transfusion satisfies the quantitative requirements for development and persistence of antigen-antibody complexes during the course of the syndrome. Our initial suggestion that therapy of this disorder should be directed at removal of residual circulating antigen by plasmapharesis rather than at removal of antibody has been upheld by observations on patients treated with this form of therapy compared to all other modes of treatment. Patients undergoing exchange transfusion, with or without concomitant platelet transfusion or corticosteroid therapy, have all had prompt reversal of hemorrhagic symptoms and return of platelets to normal whereas other forms of treatment have been relatively ineffective. All patients were exposed to antigen one or more times prior to the exposure that caused PTP and 2 patients had more than one episode of PTP after an interval between the initial episode and recurrence that allowed for decay of residual antibody. Measurements of the association constants of the responsible antibodies indicate that only a complex between high-affinity antibodies ($Ka > 10^7$) in complex with Pl^{A1} antigen have the unique potential for adsorption by Pl^{A1}-negative platelets. The Pl^{A1} antigen has been found to be proteinaceous in contrast to the characteristic carbohydrate structure of red cell antigens. The antigen has also been linked with platelet function in that patients with a congenital platelet dysfunction, thrombasthenia, do not express Pl^{A1} antigen on their platelets. The PTP syndrome has been valuable in elucidating potential mechanisms of autoimmunity, in relating platelet structure to function, and establishing quantitative aspects of immunologic reactions leading to destruction of platelets.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 51,000-23 CHB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Immunology of Blood Cell Deficiencies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Raphael Shulman	Chief	CHB, NIAMDD
Other:	James V. Jordan, Jr.	Clinical Associate	CHB, NIAMDD
	Ellen Goldfarb	Biologist	CHB, NIAMDD

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIAMDD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Autoimmunity, drug antibodies, platelet antigens, isoimmunization, transplantation antigens, hepatitis, hemagglutination tests, thrombocytopenia, blood coagulation inhibitors.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

To study the nature of immunologic reactions which result in formation of antibodies against autologous blood cells, and to determine the significance of immunity in development of "idiopathic" blood cell deficiency states. Of special interest are the biochemical reactions which result in formation of complexes between cells, antibodies, and drug haptenes and the physiologic processes which result in sequestration of cells with attached antibodies. In recent years our work on identifying inherited leukocyte and platelet isoantigens has led to a study of the significance of these antigens in hetero- and homo-transplantation. Immunologic techniques have been used to develop a number of clinically practical diagnostic tests for certain infectious disease and drug addiction.

Methods Employed:

Techniques of quantitative immunochemistry, including preparation and physiocochemical characterization of purified antibodies and antigens, microanalyses for nitrogen, histamine, and alkaloid drugs quantitative measurements of complement fixation, cellular agglutination and precipitin reactions, immunoelectrophoresis, methods of provoking antibody responses in man and animals, and isotopic and fluorescent labeling applied to antigens and antibodies. Tissue culture techniques, red cell agglutination and techniques and lymphocyte transformation tests, including radioautography. Methods of hemagglutination involving attachment of various antigens to erythrocytes by chemical coupling.

Publications:

Shulman, N.R. and Jordan, J.V., Jr.: Platelet Immunology. In Textbook of Hemostasis and Thrombosis. In press, 1982.

Shulman, N.R. and Jordan, J.V., Jr.: Post transfusion purpura. In Textbook of Hemostasis and Thrombosis. In press, 1982.

Shulman, N.R. and Jordan, J.V., Jr.: Isoimmune neonatal thrombocytopenia INT). In Textbook of Hemostasis and Thrombosis. In press, 1982.

Shulman, N.R. and Jordan, J.V., Jr.: Platelet-specific, HLA, and erythrocyte antigens in platelet transfusion and therapy. In Textbook of Hemostasis and Thrombosis. In press, 1982.

Shulman, N.R. and Jordan, J.V., Jr.: Idiopathic thrombocytopenic purpura. In Textbook of Hemostasis and Thrombosis. In press, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 51,001-23 CHB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Raphael Shulman	Chief	CHB, NIAMDD
Other:	James V. Jordan, Jr.	Clinical Associate	CHB, NIAMDD

COOPERATING UNITS (if any)

J. Axelrod	Laboratory Chief	LCS, NIMH
N. Davenport	Senior Investigator	CB, NHLBI

LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIAMDD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hemophilia, coagulation mechanism, clotting factor assays, clotting factor purification, platelet physiology, platelet metabolism, pharmacology o platelet-inhibitory drugs.

Project Description:

Objectives:

Study of the reactions and interactions of coagulation factors *in vitro* and *in vivo* to define further the nature of the blood coagulation mechanism, to determine factors of significance in the pathogenesis of diseases of hemorrhage and thrombosis, and to develop better forms of therapy for these diseases.

Methods Employed:

Methods of protein purification and characterization, techniques of enzymology applied primarily to proteolytic enzymes and their inhibitors, kinetic analyses of enzyme reactions, procedures for quantitative measurement of various clotting factors, pharmacologic and physiologic techniques applied in man and animals, and assessment of metabolic pathways of blood cells with radioactivity labeled substrates.

Publications:

Hotchkiss, A., Jordan, J.F., Hirata, F., Shulman, N.R. and Axelrod, J.: The dissociation of phospholipid methylation and human platelet function. *Biochem. Pharmacol.* In press, 1981.

Goldstein, R.E., Davenport, N.J., Capurro, N.L., Lipson, L.C., Bonow, R.O. Shulman, N.R. and Epstein, S.E.: Relative effects of sulfinpyrazone and ibuprofen on canine platelet function and prostaglandin-mediated coronary vasodilation. *J. Cardiovasc. Pharmacol.* 2:399-409, 1980.

Davenport, N., Goldstein, R.E., Capurro, N., Lipson, L.C., Bonow, R.O., Shulman, N.R. and Epstein, S.E.: Sulfinpyrazone and aspirin increase epicardial coronary collateral flow in dogs. *Am. J. Cardiol.* 47:848-854, 1981.

Markovic, O.S. and Shulman, N.R.: Megakaryocyte maturation indicated by methanol inhibition of an acid phosphatase shared by megakaryocytes and platelets. *Proceedings of Megakaryocytes In Vitro Symposium.* Elsevier-North Holland, In press, 1981.

Shulman, J.D., Mudd, S.H., Lanvader, L., Shulman, N.R.: Postpartum thrombophlebitis pyradoxene-non-responsive homocystinurea during treatment with anti-platelet agents. *Blood* Letter to the Editor, in press, 1981.

Shulman, N.R. and Jordan, J.V.,Jr.: Platelet Dynamics. In *Textbook of Hemostasis and Thrombosis*. In press, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 51,002-08 CHB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Mechanisms of Hormone and Transmitter Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Harvey B. Pollard	Senior Investigator	CHB, NIAMDD
Other:	Christopher J. Pazoles	Senior Staff Fellow	CHB, NIAMDD
	Carl E. Creutz	Senior Staff Fellow	CHB, NIAMDD
	Mark Levine	Research Fellow	CHB, NIAMDD
	Janet H. Scott	Guest Worker	CHB, NIAMDD
	Velia Fowler	Guest Worker	CHB, NIAMDD
	Samuel S. Stopak	Guest Worker	CHB, NIAMDD
	Oren Zinder	Visiting Scientist	CHB, NIAMDD
	Avner Ramu	Visiting Scientist	CHB, NIAMDD

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIAMDD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.25	5.25	1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Exocytosis of neurotransmitters, hormones and other cell secretory products is believed to occur by calcium-dependent movement of the secretory vesicle to the cell surface and subsequent fusion of the secretory vesicles with the plasma membrane. We have discovered a calcium-sensitive linkage of secretory vesicles to the cytoskeletal protein actin. We have found that the calcium binding protein synexin binds the inside of the plasma membrane as well as the secretory vesicle membrane. Membranes drawn together by this protein were demonstrated to fuse together when exposed to arachidonic acid at concentrations comparable to those present in stimulated cells. Working with isolated adrenal medullary cells we have found that epinephrine secretion appears to depend upon an energy dependent chemiosmotic lysis of the secretory vesicle.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

The object of our work has been to discover the molecular bases for regulation of cellular secretion of hormones and transmitters by exocytosis. Our approach has been to search for factors mediating calcium action, membrane-cytoskeletal interactions, the formation of complexes between secretory vesicles and plasma membranes and the final breakage (fission) of this complex leading to the ultimate release event of exocytosis.

Methods Employed:

The methods we have employed include cell fractionation, ultracentrifugation, light and electron microscopy, enzymology, scintillation counting, viscometry, light scattering, fluorescence spectroscopy and nuclear magnetic resonance.

Major Findings:

1. Interaction of the secretory vesicle with the cytoskeleton.

Actin-containing cytoskeletal and/or contractile elements have been proposed to be instrumental in the translocation of secretory vesicles to the plasma membrane during calcium-mediated exocytosis of secretory products from cells. We have studied the interaction of F-actin with the secretory vesicles from the adrenal medulla (chromaffin granules) by low shear falling ball viscometry and by cosedimentation. Purified chromaffin granule membranes bind purified rabbit skeletal muscle F-actin and cause large increases in the viscosity of F-actin under conditions that may approximate the resting intracellular environment (low salt, neutral pH, 1-2 mM $MgCl_2$, $[Ca^{++}]_{free} \simeq 10^{-8}$ M, 30°C). The actin-binding and cross-linking activity of the membranes is thermolabile (2', 100°C) and trypsin-sensitive, suggesting that F-actin interacts with a protein component in the chromffin granule membrane. Prior extraction of the membranes with 1 MKCl-3.5 M urea, but not 1 M KCl alone, also abolishes the actin-binding and cross-linking activity of the membranes. This could be due to inactivation of the actin-binding component by urea or to extraction of a tightly bound extrinsic membrane protein responsible for attachment of actin to the membrane. Raising the free calcium ion concentration to micromolar levels reversibly inhibits the increases in viscosity of membranes plus F-actin, but has no effect on the viscosity of F-actin alone. The free calcium ion concentration that results in the half-maximal inhibition is 2.6×10^{-7} M. Regulation of the interaction of F-actin with chromaffin granule membranes by free calcium ion concentrations that are thought to control secretion *in vivo* suggests that actin may indeed play a role in exocytosis. We propose that interactions between chromaffin granule membranes and actin represent naturally occurring associations of chromaffin granules with the cytoskeleton and that calcium-induced solation of this actin-chromaffin granule gel is necessary for organelle movement to the plasma membrane.

2. Interaction of the secretory vesicle with the plasma membrane: A process mediated by synexin?

Synexin is a calcium binding protein isolated from adrenal medullary and other tissues that binds to chromaffin granules and causes them to aggregate in a calcium dependent fashion. On the basis of these properties we have suggested that this protein might be involved in mediating calcium action during exocytosis. Previous work has demonstrated calcium dependent binding synexin to chromaffin granules and this year we demonstrated that plasma membrane fragments from chromaffin cells isolated on polylysine coated polyacrylamide beads also exhibit calcium dependent binding of synexin. Synexin was found to bind in a calcium dependent manner to beads coated with either plasma membranes or chromaffin granule membranes, but not at all to beads coated with erythrocyte membranes or uncoated beads. The synexin binding to plasma membrane coated beads showed a specific requirement for calcium ($K_{\frac{1}{2}} \sim 200\mu M$) and was insensitive to other divalent cations such as magnesium, strontium^{2} and barium. Synexin binding to either plasma membrane or granule membrane coated beads was saturable, partially reversible by EGTA and was directly observed by SDS polyacrylamide gel electrophoresis. The demonstration that synexin binds to plasma membrane as well as granule membranes in a similar calcium dependent manner satisfies a critical requirement for a protein presumed to be involved in the regulation of membrane recognition and fusion during exocytosis.

3. Fusion of membranes brought into contact by synexin.

When isolated chromaffin granules were aggregated by synexin and then exposed to cis-unsaturated fatty acids at 37°C they fused together to form large vesicles. The fusion was monitored by phase and electron microscopy and by turbidity measurements on the granule suspension. Arachidonic acid was the most effective fusogen, while trans-unsaturated fatty acids, saturated fatty acids, detergents or lysolecthin were inactive. During fusion some of the epinephrine of the granules was released but the soluble core proteins remained trapped in the resulting vesicles. These vesicles swelled to enclose the maximum volume. Although this swelling could be inhibited by increasing the osmotic strength of the medium, it did not appear to depend on the chemiosmotic properties of the granule membranes as it was not influenced by ATP, a proton ionophore or an anion transport inhibitor.

The regulators of this in vitro fusion -- Ca^{2+}, synexin and free, cis-unsaturated fatty acids--may be present in the cytoplasm of the chromaffin cell when it is stimulated to release epinephrine and granule proteins by exocytosis. Therefore, this fusion event may be the same that occurs between chromaffin granules undergoing compound exocytosis.

4. Catecholamine transport into chromaffin granules

Catecholamine transport into chromaffin granules is ATP dependent, but the charge of the transported species is not known. To investigate which form of catecholamine is recognized by the transport system and transported, we have used a newly synthesized compound, dimethylepinephrine (DiME). DiME is permanently charged by virtue of 3 methyl groups on its nitrogen. We discovered that DiME competitively inhibits uptake of tritiated norepinephrine with a K_I of approximately 2 mM. Tritiated DiME (^{3}H DiME) is not transported into chromaffin granules. Binding of ^{3}H DiME to chromaffin granules can be displaced by norephinephrine or unlabelled DiME, and is not temperature dependent, ATP dependent, nor reserpine sensitive. Areas under investigation include characterization of DiME binding to chromaffin granules and granule membranes, effect of DiME on catecholamine release from intact granules and granule ghosts, effect of granule charge and pH of the medium on DiME binding, and the effect of reserpine on norepinephrine ^{3}H DiME competition for granule membrane binding sites.

5. Chemiosmotic processes underlying secretion from isolated chromaffin cells

Dissociated chromaffin cells from bovine adrenal medulla were stimulated to secrete epinephrine and dopamine-b-hydroxylase (DBH) with a variety of secretagogues in a study designed to test the hypothesis that the chemiosmotic lysis reaction of isolated chromaffin granules might in some way be related to the mechanism of release during exocytosis. Increasing the osmotic strength of the incubation medium with either NaCl or sucrose led to suppression of secretion of epinpehrine from the cells regardless of whether secretion was induced with veratridine or acetylcholine. Suppression of secretion was approximately exponential with respect to osmotic strength. We also found that epinephrine secretion occurred only if the medium contained a permeant anion such as chloride. For example, secretion induced by veratridine was suppressed when Na isethionate replaced NaCl in the medium. In an extensive study with different monovalent anions we found that veratridine supported epinephrine secretion according to the following activity series: Br^-, I^-, NO_3^- >methylsulfate, SCN^->Cl> acetate>>Jisethionate. A similar series was observed with A23187 as agonist. Anion transport inhibitors such as probenecid and pyridoxal phosphate also inhibited secretion while the stilbene disulfonates were inactive. Secretion of catecholamines, induced by veratridine or nicotine, a cholinergic agonist, was suppressed when NaCl in the medium was replaced by isosmotic sucrose and unexpectedly low levels of DBH were observed in some cases. Inhibitors of ATP synthesis such as carbonyl cyanid p-trifluoromethoxy-phenyl-hydrazone (FCCP), KCN, rotenone and azide blocked epinephrine secretion effectively, as did 2-deoxyglucose, an inhibitor of glucose utilization. In summary, these properties of chromaffin cells resembled the properties of isolated chromaffin granules incubated in ATP and Cl^-, not only in terms of relative sensitivity to different anions in the medium and of apparent requirements for metabolic energy. We therefore have interpreted our data to indicate that a close mechanistic relationship may exist between the release event in exocytosis from chromaffin cells and the chemiosmotic lysis reaction characteristic of isolated chromaffin granules.

Publications:

Creutz, C.E., Pazoles, C.J. and Pollard, H.B.: Immunohistochemical and biochemical studies on synexins of diverse tissues. In Siegel, F.L., *et al*.(Eds.) Calcium-binding Proteins: Structure and Function. North Holland, Elsevier, 1980, pp. 78-80.

Pollard, H.B., Creutz, C.E., Pazoles, C.J. and Scott, J.H.: Fusion and fission processes in exocytosis: Possible roles for synexin and osmotic lysis in the two events, In Bailey, G.W. (Ed.), Proceedings of the 38th Annual Meeting of the Electron Microscopy Society of America. Baton Rouge, Claitor's, 1980, pp. 594-597.

Pazoles, C.J., Creutz, C.E. and Pollard, H.B.: Evidence for direct coupling of proton and anion transport in chromaffin granules. Ann. N.Y. Acad. Sci. 358:354-355, 1980.

Publications:

Ramu, A., Pazoles, C.J., Creutz, C.E. and Pollard, H.B.: Catecholamines transport by isolated chromaffin granules. J. Biol. Chem. 256:1229-1234, 1981.

Pollard, H.B.: X-ray diffraction of membranes and viruses. In Tiffany, J.M. and Blaugh, H.M.(Eds.): Cell Membranes and Viral Envelopes. New York, Academic Press, 1980, pp. 141-173.

Pazoles, C.J., Creutz, C.E., Ramu, A. and Pollard, H.B.: Permeant anion activation of MgATPase activity in chromaffin granules. J. Biol. Chem. 255:7863-7869, 1980.

Creutz, C.E. and Pollard, H.B.: A biophysical model of the chromaffin granule. Biophys. J. 31:255-270, 1980.

Pollard, H.B., Stopak, S.S., Pazoles, C.J. and Creutz, C.E.: A simple and novel method for radiometric analysis of glucose utilization by adrenal chromaffin cells. Anal. Biochem. 110:424-430, 1981.

Pollard, H.B., Pazoles, C.J., Creutz, C.E. and Zinder, O.: Role of intracellular proteins in the regulation of calcium action and transmitter release during exocytosis. In Levy, A., et al (Eds.): Monographs in Neural Science. New York, Karger, 1980, Vol. 7, pp. 106-116.

Creutz, C.E.: Cis-unsaturated fatty acids induce the fusion of chromaffin granules aggregated by synexin. J. Cell. Biol. In press, 1981.

Pollard, H.B., Creutz, C.E., Pazoles, C.J., Scott, J.H. and Fowler, V.: Calcium dependent regulation of chromaffin granule movement, membrane contact and fusion during exocytosis. Cold Spring Harbor Symp. Quant. Biol. 46. In press, 1981.

Pollard, H.B., Pazoles, C.J. and Creutz, C.E.: Mechanism of calcium action and release of vesicle bound hormones during exocytosis. Recent Progress in Hormone Research 37. In press, 1981.

Project Description:

Objectives:

To study normal lysosomal function as well as the biochemical defects in genetic disorders of such function, as in mucopolysaccharidoses and mucolipidoses, and to use the findings for medical assistance to the patients and their families.

Methods Employed:

Conventional biochemical techniques, such as enzyme purification, preparation of antibodies, receptor isolation, use of radioisotopes, gel electrophoresis and fluorography, preparation of mRNA and cell-free translation; culture of human fibroblasts from normal human individuals and from patients with genetic diseases, as well as culture of cells from other species.

Major Findings:

1. The previous finding of abnormal lysosomal enzymes in I-Cell disease has been extended to pseudo-Hurler polydystrophy (Mucolipidosis III). β-Hexosaminidase synthesized by fibroblasts derived from mucolipidosis III patients has very low but detectable phosphate content; it is secreted from the cells rather than incorporated into lysosomes, and it is not processed to the mature form. Normal β-hexosaminidase can be internalized by Mucolipidosis III cells and processed.

2. The "precursor piece" which is removed during maturation of β-hexosaminidase α- and β- chains remains associated with the mature enzyme (presumably by disulfide bonds) in cultured rat basophilic leukemia cells and mouse macrophages (P388-D1). This can be observed by first subjecting the mature enzyme to polyacrylamide gel electrophoresis in denaturing but non-reducing conditions; isolation of the β-hexosaminidase chains, followed by reduction and re-electrophoresis, allows the separation of a fragment of about 14,000 molecular weight. This fragment is phosphorylated in rat basophil leukemia cells.

3. Biosynthesis of β-hexosaminidase has been studied in fibroblasts of two clinically normal individuals who have no circulating β-hexosaminidase A activity. The pattern of newly-formed β-hexosaminidase was that expected of Tay-Sachs heterozygotes and the cause of the plasma enzyme deficiency is not known (collaborative with Dr. Thomas).

4. The fate of β-galactosidase internalized by Chinese Hamster Ovary cells has been studied by fluorescence and electron microscopy (collaborative with Drs. Willingham and Pastan). The enzyme is concentrated in coated pits; from there, it proceeds via uncoated vesicles to a central (Golgi/GERL) area of the cell, where it is seen in small coated vesicles, and finally it reaches lysosomes. The transit time from coated pits to lysosomes at 37° is 15 - 45 minutes.

5. A canine patient with a storage disease has been brought to our attention. Preliminary studies on cultured skin fibroblasts suggests that the disorder may be related to Mucopolysaccharidosis I.

6. The cell-free translation of β-hexosaminidase has been undertaken, using human fibroblast RNA and a rabbit reticulocyte translation system. A polypeptide immunologically identifiable as the α-chain of the protein has been found among the translation products.

Significance to Biomedical Research and the Program of the Institute: This project, begun in 1967, has led to the elucidation of biochemical defects and to the development of precise diagnostic methods for a major class of genetic diseases. The work has led to many new questions: of cell biology (how are newly formed enzymes translocated from the site of synthesis to lysosomes); of enzymology (how are lysosomal enzymes modified post-translationally); of genetics (what is the structure of the DNA encoding lysosomal enzymes, and what are the changes associated with the lysosomal storage diseases); of clinical medicine (how can an effective therapy be developed).

Proposed Course:

1. To isolate the "precursor piece" of β-hexosaminidase and study its function. Is it essential for recognition of the enzyme by the phosphorylating system, or for its packaging into lysosomes?

2. To extend the morphological studies of β-galactosidase internalization to analogous studies of the receptor, and to biochemical studies using cell fractionation. Is the receptor internalized with the ligand and if so, where do the two part company? Where and how does endogenous enzyme bind to the receptor?

3. To continue study of the canine disorder, and determine how closely it resembles a human lysosomal storage disease. If further biochemical studies confirm our expectations, to develop a colony of dogs of which the affected members could be used for experimental therapies.

4. To improve the *in vitro* translation system of β-hexosaminidase so that it can be used as an assay for mRNA, with the eventual goal of cloning the genes for β-hexosaminidase.

Publications:

Sando, G.N., and Karson, E.M.: p-Isothiocyanatophenyl 6-Phospho-α-D-mannopyranoside Coupled to Albumin. A Model Compound Recognized by the Fibroblast Lysosomal Enzyme Uptake System. 1. Chemical Synthesis and Characterization. Biochemistry 19: 3850-3855, 1980.

Karson, E.M., Neufeld, E.F., and Sando, G.N.: p-Isothiocyanatophenyl 6-Phospho-α-D-mannopyranoside Coupled to Albumin: A Model Compound Recognized by the Fibroblast Lysosomal Enzyme Uptake System. II. Biological properties. Biochemistry 19: 3856-3860,1980.

Neufeld, E.F.: Recognition and Processing of Lysosomal Enzymes in Cultured Fibroblasts. In Callahan, J.W. and Lowden, A.J. (Eds): Lysosomes and Lysosomal Storage Diseases. New York, Raven Press, 1981, pp. 115-129.

Myerowitz, R., and Neufeld, E.F.: Maturation of α-L-Iduronidase in Cultured Human Fibroblasts. J. Biol. Chem. 256: 3044-3048, 1981.

Frisch, A., and Neufeld, E.F.: Limited Proteolysis of the β-Hexosaminidase Precursor in a Cell-free System. J. Biol. Chem. in press.

Neufeld, E.F.: The Receptor for Lysosomal Enzymes on the Surface of Cultured Fibroblasts. In Middlebrook, J. and Kohn, L. (Eds.): Relationship Between Toxins and Receptors, in press.

Wasteson, A., and Neufeld, E.F.: Iduronate Sulfatase from Human Plasma. Methods in Enzymology, Vol. 83, in press.

Thomas, G.H., Raghaven, S.S., Kolodny, E.H., Frisch, A., Neufeld, E.F., O'Brien, J., Reynolds, L.W., Miller, C.S., Shapiro, J., Kazazian, H., Jr., and Heller, R.H.: Nonuniform Deficiency of Hexosaminidase A in Tissues and Fluids of Two Unrelated Individuals. Pediatric Research, in press.

Neufeld, E.F., and McKusick, V.A.: Disorders of Lysosomal Enzyme Synthesis and Localization: I-Cell Disease (Mucolipidosis II) and Pseudo-Hurler Polydystrophy, (Mucolipidosis III). In The Metabolic Basis of Inherited Disease, Basis of Inherited Disease, Stanbury, J.B., Wyngaarden, J.B., Fredrickson, D.S., Goldstein, J.L., Brown, M.S. (Eds.): New York, McGraw-Hill, 5th Edition, in press.

McKusick, V.A., and Neufeld, E.F.: The Mucopolysaccharide Storage Diseases. In The Metabolic Basis of Inherited Disease, Stanbury, J.B., Wyngaarden, J.B., Fredrickson, D.S., Goldstein, J.L., Brown, M.S. (Eds.): New York, McGraw-Hill, 5th Edition, in press.

Neufeld, E.F.: Recent Studies on the Maturation of Lysosomal Enzymes. Proceedings of the International Symposium on Inborn Errors of Metabolism, (Interlaken, 1980), in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52002-04 GBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Role of Cell-Surface Antigens in Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rafael D. Camerini-Otero	Senior Surgeon	GBB NIADDK
Other:	Richard A. Anderson	Staff Fellow	GBB NIADDK

COOPERATING UNITS (if any)
Dr. Dinah Singer, Transplantation Biology Section, Immunology Branch, NCI
Dr. Jonathan Seidman, Gene Organization and Control Section, Laboratory of Molecular Genetics, NICHD

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.50	0.50	0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have cotransformed mouse Ltk^- cells with herpes simplex virus thymidine kinase gene and several DNA clones from pig and mouse genomic lambda libraries that have hybridized to an HLA (human histocompatibility) cDNA clone. Several of these cotransformants appear to coexpress the thymidine kinase gene and genes from the major histocompatibility complexes of pig and mouse.

Project Description:

Objectives:

To understand the role of cell-surface antigens in mammalian development.

Methods Employed:

Recombinant DNA techniques, DNA-mediated gene transfer, somatic cell culture, and immunochemical methods.

Major Findings:

1. J. Seidman and his colleagues selected several lambda recombinant DNA clones from a d haplotype mouse genomic library that hybridized to a cDNA clone for an human major histocompatibility antigen (HLA). Six independent clones selected from the first screening were transferred with the herpes simplex virus thymidine kinase gene into Ltk$^-$ (tk deficient, k haptotype) mouse cells. Using several criteria, one of the cotransformants appears to be expressing a d haplotype antigen.

2. Similar experiments were carried out with a putative SLA (pig histocompatibility antigen) recombinant clone obtained in a similar manner as mentioned above from a miniature swine lambda genomic library (D. Singer). Preliminary experiments also indicate coexpression of this antigen and herpes thymidine kinase.

Significance to Biomedical Research and the Program of the Institute:

This project will lead to a better understanding of the structure and function of cell-surface and histocompatibility antigens.

Proposed Course:

To study the structure and function of the genes coding for mammalian histocompatibility antigens.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 52003-03 GBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Structure of the Methionine Initiator tRNA Genes in the Human Genome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael A. Zasloff	Senior Surgeon	GBB	NIADDK
Other:	Tomas Santos	Visiting Fellow	GBB	NIADDK
	Paul-Henri Romeo	Guest Worker	GBB	NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The initiator methionine tRNA genes previously isolated from a recombinant phage library of human fetal liver DNA have been studied in greater detail. The functional states of the genes have been assayed in a newly described *in vitro* transcription system and *in vivo*, by insertion into a recombinant SV40 chromosome. Our studies have demonstrated that a variant tRNA_i^{met} gene, containing a base substitution in a sequence highly conserved in both prokaryotes and eukaryotes is not processed efficiently to a mature species *in vitro* and *in vivo*.

Project Description:

Objectives:

1. To determine the "functional state" of the several human $tRNA_i^{met}$ genes isolated.

2. To define features of the DNA sequence of the $tRNA_i^{met}$ genes which relate to expression.

Methods Employed:

All current methods in nucleic acid research, including DNA cloning, RNA and DNA sequencing, and filter hybridization methods.

Major Findings:

1. Each of the two human $tRNA_i^{met}$ genes previously isolated can be transcribed with about equal efficiency in an *in vitro* system derived from human KB cells.

2. A method has been developed to purify the crude KB cell extract free of endogenous inhibitors, yielding a preparation capable of accurate nucleolytic processing of the $tRNA_i^{met}$ precursor species. The nature of the inhibitor has not been determined.

3. In the *in vitro* system, the primary transcript of the normal $tRNA_i^{met}$ gene is processed in an ordered series of endonucleolytic reactions: The 5' leader sequence is cleaved, followed by the 3' trailer.

4. The 'mutant' $tRNA_i^{met}$ gene, containing a G to T substitution at position 57, highly conserved in all prokaryotic and eukaryotic tRNAs, is not processed to the mature $tRNA_i^{met}$. A precursor accumulates in vitro in which the 5' leader has been trimmed to the mature sequence, but containing a totally intact 3' trailer.

5. To assay the activity in the mutant $tRNA_i^{met}$ gene *in vivo*, a recombinant SV40 virus was constructed in which the gene was inserted behind the promoter of the late region of the virus, replacing almost the entire normal late region. After infection of African green monkey kidney cells with this recombinant along with a helper SV40 virus, RNA was recovered from infected cells late in infection, and analyzed by standard methods. A predominant RNA accumulated, representing about 30% of the low molecular weight RNA recovered from the cytoplasm of these cells, and was shown to be a $tRNA_i^{met}$ precursor transcribed from the variant gene. The study represents (a) the domonstration of a first tRNA gene transcribed from a eukaryotic vector, (b) proof that a point mutation in a considered portion of the gene does not lead to inactivation at the level of transcription, (c) demonstration of the defective processing *in vivo*, (d) suggestion of the importance of RNA processing as a "proofreading" mechanism protecting the eukaryotic organism from mutational errors within the tRNA gene family.

6. The 5' and 3' flanking regions of both normal and variant $tRNA_i^{met}$ genes have been deleted to various extents by in vitro recombinant DNA methods. Data demonstrate that the control regions for the human $tRNA_i^{met}$ genes lie within the mature tRNA coding sequence.

Significance to Biomedical Research and the Program of the Institute:

The project attempts to understand the molecular basis of specific gene expression in the human genome and the mechanisms responsible for the origin of the $tRNA_i^{met}$ gene in the genome.

Proposed Course:

1. In vitro detection construction of the $tRNA_i^{met}$ genes will be continued to more precisely define the 5' and 3' boundaries of the control regions of each gene.

2. Fractionation of the partially purified KB cell extracts will continue, in order to characterize components (in addition to RNA polymerase) needed for specific transcription of the $tRNA_i^{met}$ gene.

3. The $tRNA_i^{met}$ gene has been fused to the herpes thymidine kinase gene, in such a manner that the control regions on the viral gene have been replaced by the human tRNA gene. We wish to explore the properties on this synthetic gene by direct injection into the germinal vesicle of the *X. laevis* oocyte, or by transfer into mouse LTK^- cells. By following the levels of viral TK enzyme the activity on the $tRNA_i^{met}$ promoter could be studied in vivo, permitting analysis of in vivo regulation of the $tRNA_i^{met}$ gene. In addition, the construction will demonstrate whether a gene like TK normally transcribed in low abundance can be produced in yields comparable to $tRNA_i^{met}$ a predominant cellular RNA.

Publications:

Santos, T., and Zasloff, M.: Comparative analysis of human chromosomal segments bearing non-allelic dispersed $tRNA_i^{met}$ genes. Cell 23: 699-709, 1981.

Zasloff, M., and Santos, T.: Reiteration frequency mapping: Analysis of repetitive sequence organization within cloned DNA fragments containing the human initiator methionine tRNA gene, Proc. Natl. Acad. Sci. USA, Vol. 77: 5668-5672, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52006-02 GBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pathophysiology of Fibrodysplasia Ossificans Progressiva and Therapeutic Intervention

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael A. Zasloff	Senior Surgeon	GBB NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fibrodysplasia ossificans progressiva (FOP) is a heritable disorder of mesenchymal cell differentiation in which heterotopic bone develops in the connective tissue surrounding skeletal muscle. In our studies on the basic cellular defect we have discovered that the cellular transformation characteristic of FOP differs from the non-genetic, non-progressive disorder, myositis ossificans traumatica. Clinical studies have demonstrated a previously undescribed primary or secondary amenorrhea in affected females, and the pathophysiology of the associated condition is currently under study. A limited clinical trial is underway to determine the efficacy of a synthetic retinoid, a drug suggested by in vitro studies to be a potentially useful agent in the treatment of FOP.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

1. To understand the cellular basis of heterotopic bone formation in fibrodysplasia ossificans progressiva (FOP).

2. To establish an appropriate course of treatment for affected individuals.

3. To define appropriate sensitive diagnostic criteria in order to follow the progress of the condition.

Methods Employed:

Tissue culture methodology; implantation of human tissue into athymic mice; techniques of diagnostic imaging including computerized axial tomography and bone scanning; routine clinical diagnostic procedures; thermography.

Major Findings:

1. All post-pubertal females with FOP have either primary or secondary amenorrhea, an association we have discovered during an extensive review of clinical histories. Since most affected individuals experience an otherwise normal pubertal progression, and retain a post-pubertal female habitus, we believe the cause of amenorrhea to be due to ovarian or uterine pathology.

2. A study was undertaken to compare the lesions of FOP with a similar metaplastic condition, myositis ossificans traumatica (MOT). MOT, like FOP, is condition in which bone elements arise from loose connective tissues surrounding skeletal muscle. MOT, however is not transmitted as a genetic condition, and is non-progressive in character. Our review of biopsy specimens of the lesions of both of these two conditions revealed a previously undescribed basic difference between FOP and MOT. In the genetic disorder, proliferating fibroblast-like cells appear to differentiate into both cartilage and bone elements. The physical organization of the lesion strongly suggests that both chondroblasts and osteoblasts arise from a common cellular precursor. In the MOT lesion, however, proliferating fibroblasts appear to differentiate solely into osteoblasts. No cartilaginous elements lie within the zone of cellular transformation. It appears that factors permitting differentiation of hard tissues from mesanchymal elements in MOT are more restrictive then in FOP. The cellular basis of this phenomenom remains obscure.

3. The children have seen treated with the synthetic retinoid, Ro 10-9359, provided by Hoffman La Roche, for a period of 6 months. The drug was considered because of its effect on the inhibition of cartilage differentiation in several systems, both in vitro and in vivo. In one child the appearance of new lesions appears to have been prevented. Further

studies will hopefully help distinguish a therapeutic effect from a spontaneous remission.

Significance to Biomedical Research and the Program of the Institute:

The project attempts to elucidate the cellular basis of a fatal inherited condition, provide rational diagnostic criteria for management, and develop an effective treatment protocol.

Proposed Course:

1. We will continue to define the natural history of the development of the metaplastic lesion.

2. The pathophysiology of the amenorrhea in FOP will be explored, and studies of sexual functions of affected males will begin.

3. The trial of Ro 10-9359 will be reviewed, and most likely will be extended to a larger scale clinical investigation.

Publications:

Schroeder, H., and Zasloff, M.A.: The spectrum of hand and foot malformations in fibrodysplasia ossificans progressiva. Johns Hopkins Medical Journal, 147: 73-78, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52007-02 GBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pseudomonas Infection in Cystic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael A. Zasloff	Senior Surgeon	GBB	NIADDK
Other:	Carmen Tejedor	Guest Worker	GBB	NIADDK

COOPERATING UNITS (if any)

LBM/NIADDK - Dr. John Foulds

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have discovered that *Pseudomonas aeruginosa* specific bacteriophage are present in high concentrations in the sputum of patients with active bronchopulmonary *P. aeruginosa* infections. At least 12 different phage have been isolated from sputum of eight patients, seven of whom had cystic fibrosis; these phage were characterized by host range sensitivity and electron microscopic morphology. We have suggested that phage might play a role in the natural history of human *Pseudomonas* infections.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

To understand the mechanism responsible for the colonization of the bronchopulmonary tree of the patient with cystic fibrosis by mucoid strains of *Pseudomonas aeruginosa*.

Methods Empolyed:

1. Growth and cultivation of pseudomonads; phage typing of pathological specimens; quantitative phage adsorption assays; isolation of bacterial mutants; characterization of bacteriophage and aeruginocins by electron microscopy, antisera inactivation, buoyant density and bacterial sensitivity spectra.

Major Findings:

1. About 12 different strains of *P. aeruginosa*-specific phage were recovered from the sputum of 8 patients, seven of whom had cystic fibrosis.

2. The phage was present at concentrations in sputum ranging between 10^3 to 10^7 particles/ml, with the corresponding concentration of *P. aeruginosa* ranging between 10^5-10^8/ml. By electron microscopy, the phage resembled previously described *Pseudomonas* phage.

3. In 3 patients studied, no serum antibodies to the phage present in their sputum could be detected, even several months after the initial identification of the phage.

4. Phage was assumed to arise in sputum from lytic growth on sensitive bacterial strains prevent in the airway, since both phage and bacteria capable of supporting lytic growth could be recovered from an individual's sputum sample.

Significance to Biomedical Research and the Program of the Institute:

These studies should extend our knowledge not only of the biological basis of the peculiar and almost pathognomonic colonization of the bronchopulmonary tree of cystic fibrosis patients with a severely pathogenic *Pseudomonas* strain, but also elucidate the complex ecological interrelationships of pseudomonads and the ubiquitous bacteriophage with which they coexist.

Proposed Course:

1. Characterization of the phage present in the airways of CF patients will continue. We would like to determine if specific strains are associated with infections in CF patients.

2. Further studies of the association of mucoid *Pseudomonas* with CF will continue. We will follow up some published studies which suggest that epithelid cells of CF individuals permit greater adherence of mucoid *Pseudomonas* compared to cells of unaffected individuals.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52008-02 GBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Gene Expression and Human Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rafael D. Camerini-Otero	Senior Surgeon	GBB	NIADDK
Other:	Richard A. Anderson	Staff Fellow	GBB	NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	1.50	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ltk$^-$ aprt$^-$ mouse L cells were transformed to the tk$^+$ phenotype with 10 nanograms of the herpes simplex virus thymidine kinase (tk) gene and 20 micrograms of several carrier DNA's (unlinked to the tk). Five transformant cell lines were studied in detail. Two lines were derived from transformations using circular pBR322 DNA as the carrier and three cell lines were obtained from transformations using circular SV40 DNA as the carrier. In all cases we were able to show that many of the restriction endonuclease fragments generated from the cellular DNA annealed to both tk and to pBR322 or SV40. In all of the cell lines some of these fragments also contain cellular DNA sequences. Thus it appears that when exogenous DNA's are introduced into these cells they are linked to each other before they are integrated into the cellular DNA. We have also been able to do a family study of contractural congenital arachnodactyly. The relationship of this condition to the Marfan syndrome, with which it can be confused, was studied by reviewing the reported incidence of the physical findings characteristic of both conditions. Criteria for distinguishing the two are being developed.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

To study the molecular control of gene expression. To use this knowledge to develop new techniques for gene purification.

Methods Employed:

Methods of molecular biology and biochemistry. Recombinant DNA methodology. Techniques of cell culture and somatic cell genetics.

Major Findings:

1. When the herpes thymidine kinase gene is transferred into mouse Ltk$^-$ cells (mouse L cells lacking thymidine kinase), tk$^+$ transformants are obtained. The nature of the carrier DNA determines the frequency of transformation. When Ltk$^-$ DNA is used the frequency is 1 transformant per 20 picograms of HSV-tk gene. When carrier DNA's with a defined sequence are used (pBR322, SV40 or M13 DNA) the frequency of transformation is one to two orders of magnitude lower.

2. The DNA from transformants obtained with pBR322 or SV40 as the carrier has been studied by the technique of Southern and northern blotting. In all cases studied many of the restriction endonuclease fragments generated from the cellular DNA of the tk$^+$ transformants anneal to both tk and to pBR322 or SV40 sequences. We interpret this to indicate that the pBR322 and SV40 sequences are covalently linked to the tk sequences.

3. Similar data obtained using restriction enzymes which do not cleave either pBR322 or SV40 and tk can be used to determine how many separate domains of the carrier (pBR322 or SV40) and the selected gene (tk) sequences there are. In all cell lines, but one, there is only one such domain. In the one exception there appear to be at least three domains. This data also indicates that there are cleavage sites for those enzymes flanking the exogenous DNA. These sites must be on the DNA from the recipient cell.

4. Restriction endonucleases that cut pBR322 or SV40 only once produce bands expected for unit length SV40 or pBR322. This suggests that multiple copies of the carrier sequences have been integrated in a head-to-tail array.

That the single-length copies of pBR322 and SV40 generated are not due to unintegrated copies of these circular molecules was ruled out in a variety of ways including electrophoretic analysis of undigested DNA, acid-phenol extracts and Hirt supernatants.

We conclude that most of the exogenous sequences exist as part of a high molecular weight structure containing the tk sequences linked to carrier DNA which is itself linked to cellular DNA.

6. For one of our cell lines the data can be incorporated into a self-consistent map of integrated sequences. In this cell line two copies of the tk gene (one complete and one partial) are linked and flanked by pBR322 sequences. The junctions between tk and pBR322 involve different regions of tk and the plasmid and both pBR322's are in the same orientation. Flanking this domain are cleavage sites for "no cut" enzymes, indicating the presence of cellular DNA on at least one side of the domain.

7. Attempts were made to "rescue" the pBR322 sequences linked to cellular or tk sequences by digesting the cellular DNA with a restriction endonuclease, ligating the fragments under conditions maximizing circularization and using the DNA to transform E. coli to ampicillin resistance. We have not isolated an "intracellular" recombinant derived from the transformants discussed above. Recently, we have repeated the same experiments with a new pBR322 derivative that can in fact be "rescued" with a reasonable efficiency. These "rescued" intracellular recombinant plasmids will allow us to ask even more detailed questions about the recombination process.

8. We have been able to carry out a family study including 4 generations and a dozen affected individuals with congenital contractural arachnodactyly (CCA) studied previously and diagnosed as having Marfan syndrome. CCA has very many similar manifestations to those found in the Marfan syndrome, including arachnodactyly, pectus excavatum or carinatum, increased incidence of mitral valve prolapse, etc. They do not seem to have the ocular findings and the incidence of serious cardiovascular disease (e.g. aortic aneurysms and dissection) seems to be not as high as in the Marfan syndrome. The relationship of this syndrome to Marfan's syndrome and similar connective tissue genetic disorders is still not clear.

Significance to Biomedical Research and the Program of the Institute:

In order to understand normal human development, developmental anomalies and genetic disorders, we must first understand the mechanisms controlling the expression of genes in mammalian cells. Our ability to manipulate genetic information and then introduce it and have it expressed in mammalian cells is a significant advance in our understanding of eukaryotic gene expression. Furthermore, the ability to introduce genes into cells will enable us to purify genes that cannot be isolated with the present technology. Many of the genes for known human genetic disorders are of this type.

Proposed Course:

1. To study the mechanisms responsible for somatic cell recombination in higher eukaryotes.

2. To investigate whether foreign DNA can be directed to specific sites in the mammalian genome.

3. To devise a method to study promoter function for polymerase II type genes.

4. To study whether DNA transferred into a mammalian cell is a mutagen and/or carcinogen.

5. To extend the ability to transform cells to other eukaryotic cells.

6. To use DNA-mediated gene transfer to purify genes that code for nonselectable functions.

7. To continue clinical studies on the Marfan-like syndromes.

Publications:

Camerini-Otero, R.D. and Zasloff, M.: Nucleosomal packaging of the thymidine kinase gene of Herpes Simplex virus transferred into mouse cells: An actively expressed single-copy gene. Proc. Natl. Acad. Sci. USA, 77: 5668-5672, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 52009-02 GBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chinese Hamster Ovary Cell Mutants with Altered Lysosomal Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	April R. Robbins	Staff Fellow	GBB NIADDK

COOPERATING UNITS (if any)

Sharon S. Krag, Biochemistry Dept., School of Public Health, Johns Hopkins University, Baltimore, Maryland

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to understand the mechanism(s) responsible for compartmentalization of acid hydrolases into lysosomes we are isolating and analyzing mutant CHO cells defective in this process. An initial step in compartmentalization involves interaction of the mannose 6-phosphate receptor with phosphorylated oligosaccharides on the hydrolases. We have examined the effects of several glycosylation defects on the ability of the oligosaccharide to serve as an acceptor of phosphate.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

To dissect the steps in lysosomal enzyme biosynthesis, modification and compartmentalization through analysis of mutants isolated from cultured cells.

Methods Employed:

Techniques of somatic cell genetics, cell fractionation, metabolic labelling, electrophoresis, enzymology and carbohydrate chemistry.

Major Findings:

1. Mutant CHO cells defective in the proteolytic processing of exogenously added human precursor α-L-iduronidase have been isolated. These mutants were shown to carry out normal processing of CHO α-L-iduronidase and β-hexosaminidase as well as of human β-hexosaminidase.

2. A mutant CHO cell originally isolated for its altered binding and uptake via the mannose 6-phosphate receptor has been shown to be defective in the biosynthesis of mannosyl phosphoryl dolichol. Thus, the mutant is unable to synthesize "high-mannose" oligosaccharides containing greater than five mannose residues. Presumably the receptor defect observed in this mutant reflects the involvement of high mannose oligosaccharides either in binding of substrate to the receptor or in the proper insertion of the receptor into the membrane.

3. We have previously shown that a CHO cell mutant which is deficient in the glucosylation of the lipid-linked oligosaccharide fails to phosphorylate oligosaccharides on its lysosomal enzymes. Instead, acid hydrolases from this mutant contain oligosaccharides of the complex type. We now have shown that prior to modification, newly synthesized acid hydrolases from the mutant contain oligosaccharides of the high-mannose type.

Significance to Biomedical Research and the Program of the Institute:

Absence of one or more acid hydrolases from the lysosomes results in lysosomal storage disease. The mechanism(s) of compartmentalization of acid hydrolases, whether endogenously synthesized or exogenously added, is unknown. Isolation and characterization of mutants altered in hydrolase compartmentalization will contribute to the understanding of this process.

Proposed Course:

1. The structure of the phosphorylated oligosaccharide on acid hydrolases isolated from the mutant deficient in biosynthesis of mannosyl phosphoryl dolichol will be examined. This structure is of interest because while the mutant cannot synthesize the type of oligosaccharide that is phosphorylated by wild type cells, acid hydrolases from this mutant bind with high affinity to the mannose 6-phosphate receptor.

2. We will compare the structures of the high-mannose oligosaccharides that serve in wild type cells as precursors of phosphorylated oligosaccharides and in the glucosylation - deficient mutant as precursors of complex oligosaccharides.

3. Mutants have been and are being isolated by selection of cells resistant to the toxicity of one lysosomotropic protein, followed by screening of the resistant cells for those unable to accumulate a second, unrelated lysosomotropic protein. In this way we hope to eliminate receptor-defective mutants, which until now have made up the majority of uptake-deficient mutants isolated by ourselves and others. The mutants isolated by this combined procedure will be analyzed.

Publications:

Robbins, A.R., Myerowitz, R., Youle, R.J., Murray, G.J., and Neville, D.M., Jr.: The mannose 6-phosphate receptor of Chinese hamster ovary cells. Isolation of mutants with altered receptors. J. Biol. Chem., in press.

Robbins, A.R. and Myerowitz, R.: The mannose 6-phosphate receptor of Chinese hamster ovary cells. Compartmentalization of acid hydrolases in mutants with altered receptors. J. Biol. Chem., in press.

Annual Report of the Digestive Diseases Branch

SUMMARY OF BRANCH ACTIVITIES

The Digestive Diseases Branch has two sections (Section on Gastroenterology and the Liver Diseases Section). The Liver Diseases Section has 2 senior physicians and 3 clinical associates; the Section on Gastroenterology has 3 senior physicians and 5 clinical associates. The Digestive Diseases Branch also has 5-10 guest investigators from other laboratories.

Detailed summaries of the activities of each section precedes the individual project reports. Both sections are engaged in investigations of basic biologic processes (e.g., hormone action, membrane transport, cellular and humoral immunology) and are attempting to apply this information to understand the pathophysiology of various disorders involving the liver and gastrointestinal tract. Both sections are also involved in attempts to improve therapy of clinical disorders such as neoplasms associated with overproduction of gastrointestinal hormones, hepatitis and fulminent hepatic failure.

Section on Gastroenterology

During this past year we have reorganized our clinical service to accomodate the increasing number of consultations requested by other physicians in the Clinical Center and the increase in the number of patients referred to our section for clinical investigations. Although this reorganization has resulted in improved patient care and more comprehensive clinical investigations, it has somewhat diminished our research productivity. Having completed the reorganization, we anticipate that our productivity will increase during the coming year.

We are in the process of following more than 30 patients with Zollinger-Ellison syndrome (gastrin-producing pancreatic neoplasm, hypergastrinemia and increased secretion of gastric acid). All of these patients are being managed using oral medications (cimetidine alone or plus an anticholinergic agent). During the past year we have reviewed all of our cases and have shown the following: 1) If secretion of gastric acid is reduced to below 10 mEq/hr, ulcers will heal and remain inactive; 2) With time, virtually all patients require an increase in the frequency or amount of medication and 3) Medical therapy does not alter the clinical course of the underlying neoplastic disease.

In the course of studying the biochemical basis of action of various pancreatic secretagogues, we found that tumor-promoting agents derived from croton oil (phorbol esters) are the most efficacious pancreatic secretagogues yet described. These phorbol esters also appear to have a unique mechanism of action in that they do not activate adenylate cyclase and do not cause mobilization of cellular calcium. During the coming year we plan to attempt to elucidate the mechanism by which phobol esters alter pancreatic acinar cell function.

Previously we have found that derivatives of various cyclic nucleotides are specific, fully competitive antagonists of the interaction of cholecystokinin (CCK) and structurally related peptides with their cell surface receptors. During this past year we have discovered a second class of CCK receptor antagonists, which are chemically unrelated to the cyclic nucleotides. Proglumide, a derivative of glutaramic acid, and benzotript, a derivative of tryptophan, can each antagonize the action of CCK in a fully competitive fashion by blocking the interaction of the peptide with its cell surface receptors. Proglumide and benzotript may have therapeutic potential in man because they are relatively inexpensive and are absorbed intact after oral administration.

Studies of the binding of ^{125}I-cholecystokinin to its cell surface receptors on various target tissues indicate that the receptor for CCK has two functionally distinct binding sites and that the nature of the alteration in target cell function depends on whether one or both of the binding sites are occupied. During the coming year we plan to develop analogues of CCK that will interact selectively with one of the two receptor binding sites.

We have developed a preparation of dispersed smooth muscle cells prepared from mammalian gastric antrum. These cells respond to a variety of gastrointestinal hormones and neurotransmitters and provide a prepartion that can be used to examine the biochemical basis of action of various agonists on smooth muscle function *in vitro*.

ATP is thought to be the chemical mediator of the so-called "purinergic nervous system". The receptors for ATP are characterized by their abilities to interact with ATP, ADP and AMP but not with adenosine. The biochemical basis of action of adenine nucleotides on their target tissues is not known. During this past year we have found that ATP, ADP and AMP are among the most effective intestinal secretagogues described and that these nucleotides interact with specific receptors on dispersed intestinal epithelial cells to activate adenylate cyclase and increase cellular cyclic AMP. Thus, ATP, ADP and AMP can interact with purinergic receptors to catalyze the conversion of intracellular ATP to cyclic AMP which, in turn, modifies the function of the target cell.

Liver Diseases Section

The Liver Diseases Section is currently conducting nine principal studies:

I. Studies of the Pathogenesis of Encephalopathy in Fulminant Hepatic Failure

A highly reproducible model of fulminant hepatic failure (FHF) has been developed in an inbred strain of rabbits. The model resembles human FHF in all important respects.

The pattern and amplitude of visual evoked potentials in the central nervous system have been monitored in rabbits via chronically implanted epidural electrodes. These potentials reflect post-synaptic neuronal activity. Repeated visual stimulation with real time computer-assisted summation increased the reproducibility of visual evoked potentials by removing background electroencephalographic activity. The recording of visual evoked potentials has been shown to be a reliable, quick, non-invasive method of quantifying neurologic changes in hepatic encephalopathy (HE). Furthermore, the pattern of visual evoked potentials associated with HE was found to be similar to that associated with coma induced by certain hypnotic agents, e.g., barbiturates and benzodiazepines, but not others, e.g., ether. As barbiturates and benzodiazepines are known to mediate their neuroinhibitory effects by interacting with post-synaptic receptors for γ-aminobutyric acid (GABA) in the central nervous system, it seems possible that putative toxins which might contribute to HE might do so by interacting with the GABA inhibitory neurotransmitter system.

To investigate GABA metabolism and the status of the GABA neurotransmitter system in HE, a highly sensitive and reproducible competitive radioreceptor assay for GABA has been set up and characterized. Studies in rabbits involving the application of this assay have shown: (i) A major source of GABA in peripheral blood is the enteric bacterial flora; (ii) plasma levels of GABA are increased by more than an order of magnitude in FHF but not in another type of metabolic encephalopathy; (iii) as the liver fails plasma levels of GABA become elevated before the onset of HE; and (iv) the density of receptors for GABA on postsynaptic neural membranes of the brain is increased in FHF. To determine whether GABA in plasma can gain access to the brain, the transport of the non-metabolizable isomer of GABA, α-aminoisobutyric acid (AIB), across the blood brain barrier was measured by a computer-assisted quantitative autoradiographic technique. Blood to brain transport of ^{14}C-AIB is minimal in normal rabbits but with liver failure it increases markedly in specific gray areas of the brain prior to the onset of HE. These findings suggest that as the liver fails plasma GABA derived from the gut crosses an abnormally porous blood-brain barrier before the onset of HE and that such GABA induces its own receptors in the brain. As GABA is the principle inhibitory neurotransmitter of the mammalian brain this hypothesis could account for the generalized neural inhibition of HE.

To determine whether changes in end-organ responsiveness could contribute to the increased sensitivity to hypnotic drugs, such as benzodiazepines, which occurs in HE, the specific binding of ^{3}H-flunitrazepam to post-synaptic neural membranes of the rabbit was studied. The number of binding sites was increased in FHF, suggesting that the benzodiazepine binding site and the GABA receptor are regulated as a single operational unit and that the increased sensitivity to benzodiazepines in HE is due to the presence of more benzodiazepine receptors permitting increased drug effect.

The mechanism by which plasma GABA concentrations become elevated in FHF has been studied in rabbits. The activity of the enzyme which catabolizes GABA in the liver, GABA-transaminase, does not decrease in FHF. However, clearance of ^{3}H-GABA from plasma in FHF is markedly decreased, suggesting that the mechanism of the elevated plasma levels is inefficient hepatic extraction.

Studies of the binding of GABA to post-synaptic membranes prepared from brains obtained at human autopsies and measurements of plasma GABA levels in patients with a variety of liver and other diseases are being conducted to determine whether the findings obtained with the rabbit model of FHF apply to man. Preliminary results indicate that the number of GABA receptors is increased in the brains of patients dying of FHF or chronic hepatocellular failure but not in patients dying from a variety of other causes. Furthermore, plasma GABA levels were found to be elevated fourfold in patients with decompensated cirrhosis without overt HE but were elevated more than tenfold in patients with cirrhosis and HE or FHF. [E. A. Jones, D. F. Schafer, P. Ferenci, J. G. Waggoner, M. Horowitz (NCI), P. Munson (NICHHD)].

II. Studies of Cellular Immune Function in Primary Biliary Cirrhosis

The role of abnormal immune mechanisms in the pathogenesis and perpetuation of the hepatobiliary lesion of primary biliary cirrhosis (PBC) is being studied. Recent studies have been designed to determine whether there is a defect in immune regulation in patients with PBC. T lymphocyte-mediated help and suppression of pokeweed mitogen-induced immunoglobulin (Ig) synthesis by cultured B lymphocytes have been quantitated *in vitro* using sensitive radioimmunoassays to measure IgG and IgM. Ig synthesis by cultures containing different T:B cell ratios, cultures containing B cells in the presence of autologous or allogeneic T cells, and cultures containing B cells and unirradiated or irradiated T cells was determined. The results suggest that there is deficient suppression of Ig synthesis in PBC due to a T cell defect. Patient control experiments indicated that the suppressor T cell defect in PBC is not secondary to chronic cholestasis or hepatocellular disease per se and that the defect exhibits specificity for PBC among liver diseases.

It has been shown that T cells can be induced to proliferate when cultured with either autologous or allogeneic irradiated B cells. These phenomena are known as the autologous and allogeneic mixed lymphoyte reactions (MLR), respectively. The recent finding by others that patients with systemic lupus erythematosus (SLE) exhibit deficiencies of both suppressor T cell function and the autologous MLR prompted studies of the MLR in PBC. For patients with PBC, the mean autologous MLR was found to be significantly less than that for controls. Thus, as in SLE, deficiencies of both suppressor T cell function and the autologous MLR are found in PBC. These deficiencies could be due to a common defect of cell interactions involved in the generation of suppressor effector cells. Such a defect could play an important role in the pathogenesis of autoimmune diseases such as PBC.

In additional studies the proliferative responses of peripheral blood mononuclear cells (PBMC) and T cells when cultured with hapten-modified stimulator cells were measured. The stimulator cells were PBMC or B cell-enriched PBMC that had been treated with trinitrobenzenesulfonic acid and irradiated. The results indicated that the primary but not the secondary proliferative response is decreased in PBC. They suggest that in PBC there is a decreased proliferative response of a subpopulation of suppressor T cells which may be responsible for a decreased recognition of exogenous antigens in association with autologous cell surface antigens. [E. A. Jones, S. P. James (NCI), C. O. Elson (NCI), and W. Strober (NCI)].

III. Studies of Protease Inhibitor Phenotypes

To study the possible relationship between intermediate deficiency states of alpha-1-antitrypsin (α_1AT), i.e. non-MM phenotypes, and the development of pulmonary disease, Pi phenotypes were determined in 52 patients with Sjogren's syndrome. The proportion of these patients who had Pi phenotypes other than MM was not significantly greater than that for populations of normal subjects. Mean values for tests of pulmonary function, including estimates of both restrictive lung disease and airway obstruction, in patients with the MM phenotype were not significantly different from corresponding means for patients with non-MM phenotypes. These findings suggest that non-MM phenotypes in patients with Sjogren's syndrome are not associated with an increased susceptibility to develop chronic obstructive pulmonary disease.

Pi phenotypes of sera from a large number of black South African patients with hepatocellular carcinoma and from age and sex matched healthy black South Africans are being determined to assess whether the incidence of any particular Pi phenotypes is increased in the patient population. [E. A. Jones and J. Vergalla].

IV. Studies of Chronic Hepatitis B Surface Antigen (HBsAg) Carriers

Patents with histologically confirmed chronic HBsAg positive hepatitis are being studied. A total of 78 patients have been evaluated and 37 followed for at least one year. Twenty to thirty percent of patients undergo spontaneous resolution of their chronic hepatitis disease activity each year (as assessed by serial determinations of serum levels of aminotransferases). In each instance the spontaneous resolution of elevated serum aminotransferase levels has been accompanied by serological changes in hepatitis B virus markers. Most striking has been the disappearance of hepatitis B e antigen ("e" or HBeAg) and seroconversion to anti-HBe concurrent with the fall to normal of serum aminotransferase levels.

These studies on the natural history of chronic type B hepatitis were initiated as a prelude to the start of trials of anti-viral agents for this condition. Two such trials have been undertaken using the antiviral agent, adenine arabinoside monophosphate (Ara-AMP). Patients selected to be treated with this agent have been carefully monitored and pre-screened to establish that they are not on the verge of a spontaneous remission. This is assured by demonstrating that patients have high and stable levels of hepatitis B virus - as assessed by serial serum determinations of HBsAg titer, HBeAg, DNA-polymerase and hepatitis B virus DNA. One such trial has been completed. Six patients with chronic type B hepatitis were treated with a ten day course of Ara-AMP. All patients had an immediate response in that the levels of circulating hepatitis B virus DNA-polymerase and DNA decreased. However, only one patient (17%) had a long lasting response - the viral markers became negative and remained negative, seroconversion from HBeAg to anti-HBe occurred and the serum levels of aminotransferases fell to normal values. The remaining five patients had only a transient response, the serum DNA polymerase and DNA levels returning to pre-treatment levels as soon as the drug was stopped. As the amount of Ara-AMP given in this first trial might have been insufficient for an optimum response to have occurred, a second study was undertaken to evaluate a 28 day course of therapy. This is a randomized controlled trial. Fifteen patients have been admitted to the study and eight have received Ara-AMP. So far, only one of the eight treated patients has had a long lasting response to therapy. In no patient in the control group has there been a loss of DNA polymerase or seroconversion from HBeAg to anti-HBe.

While effective antiviral treatment of chronic type B hepatitis remains an important goal, treatment of acute, uncomplicated type B hepatitis with antiviral agents is probably not feasible since maximal DNA polymerase activity, which is indicative of maximal viral replication, occurs before clinical symptoms become severe or serum aminotransferase levels reach peak values.

An epidemiological study of hepatitis B viral markers in an isolated Canadian Inuit community has indicated that hepatitis B virus infection was common in the community 20-30 years ago and that infection occurred predominantly during infancy, either as a result of maternal-infant transmission or post-natal exposure. During the past 20 years transmission of type B hepatitis to children in this community has become uncommon.

The mechanism by which some individuals who become infected with the hepatitis B virus fail to clear the virus and develop chronic type B hepatitis is being investigated. Failure to detect anti-HBs in chronic HBsAg carriers may be due to complexing of anti-HBs by excess circulating HBsAg or to a defect in the synthesis of anti-HBs. To examine the latter of these possibilities pokeweed mitogen-induced synthesis of anti-HBs, anti-HBc, IgG and IgM by peripheral blood mononuclear cells from HBsAg carriers and controls has been measured *in vitro* using sensitive assays. The results indicate that lymphocytes from HBsAg carriers have an unimpaired ability to synthesize both IgG and IgM and are able to synthesize detectable amounts of anti-HBc. However, they do not synthesize measurable amounts of anti-HBs *in vitro* despite continuous antigenic exposure to HBsAg *in vivo*. The potential role of abnormal regulation of the immune response in the pathogenesis of chronic type B hepatitis is also being studied. T lymphocyte-mediated help and suppression of pokeweed mitogen-induced immunogloblin (Ig) synthesis by cultured B lymphocytes has been quantitated *in vitro* using enzyme-linked immunoassays (ELISA) to measure IgG and IgM. At a T:B cell ratio of approximately 1:1 maximum

Ig synthesis by cultures of cells from normal individuals occurs (helper effect) and at higher T:B cell ratios progressively less Ig is synthesized (suppressor effect). In contrast to cultures of cells from normal individuals, cultures of cells from many patients with chronic type B hepatitis continue to synthesize progressively larger amounts of Ig with increasing T:B cell ratios, indicating a defect in suppressor cell function in these patients. Following irradiation of T cells to selectively destroy suppressor T cells there appeared to be no difference in Ig synthesis by cultures of cells from patients with chronic type B hepatitis and normal subjects, indicating that helper T cell function was normal in the patients. To investigate the nature of the suppressor cell defect in chronic type B hepatitis Ig synthesis by cultures containing complementary subsets of allogeneic immune cells from patients with chronic type B hepatitis and normal individuals is being determined. The patients being studied are from a group with chronic type B hepatitis who will subsequently receive therapy with one or more antiviral agents such as Ara-AMP. Restudy of those patients who have responded successfully to therapy will permit identification of those defects of immune regulation which contribute to the development of chronic type B hepatitis and distinguish those which arise secondary to chronic hepatitis. [J. H. Hoofnagle, S. Straus (NIAID), G. M. Dusheiko, G. Y. Minuk, W. G. E. Cooksley, J. Gerin (Georgetown University), R. Johnson (NIAID), R. H. Purcell (NIAID), and E. A. Jones].

V. Controlled Trial of Chlorambucil Therapy in Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is characterized by slowly progressive intrahepatic cholestasis due to a chronic non-suppurative destructive cholangitis involving the septal and larger interlobular bile ducts. When symptomatic the disease appears to be uniformly fatal. The disease is associated with multiple defects of immunologic function suggestive of autoimmunity as well as hepatic copper overload. Trials of corticosteroids and azathioprine have not shown that either of these agents effectively inhibit the progression of the disease or increase survival. Trials of penicillamine suggest that there may be some improvement in the mortality if the drug is given to patients with advanced stages of the disease. However, penicillamine treatment in this disease is associated with a high incidence of serious side effects and many patients cannot tolerate the drug. Because certain other chronic inflammatory autoimmune diseases appear to respond favorably to alkylating agents but not to azathioprine, a controlled randomized trial of an alkylating agent for the treatment of PBC has been initiated. The alkylating agent chosen for the trial was chlorambucil rather than cyclophosphamide because of the less severe toxicity associated with the use of the former drug. So far 16 patients have been admitted to the trial. Each patient undergoes a comprehensive medical evaluation prior to randomization and again after being in the trial for one and two years. Each evaluation includes determinations of indices of cholestasis and hepatocellular function, serum levels of lipoproteins, autoantibodies, immune complexes and bile acids, and a needle biopsy of the liver. In those receiving the drug marked lymphocytopenia is induced. The dose is titrated so that a polymorphonuclear leucocyte count of at least $1000/mm^3$ is maintained. Five patients receiving the drug have had follow-up liver biopsies which show a marked decrease of inflammatory activity.

VI. Studies of Chronic Non-A, Non-B Hepatitis

Patients with well-defined chronic non-A, non-B hepatitis are being evaluated. The natural history of this condition is being studied in collaboration with Dr. Harvey J. Alter (NIH Clinical Center Blood Bank). Patients with HBsAg negative post-transfusion hepatitis whose serum levels of aminotransferases remain elevated for more than six months are being admitted for a thorough assessment. Studies include HLA typing, and serological assays for non-A, non-B antigens and antibodies. Unless contraindicated a needle biopsy of the liver is obtained and is subjected to light microscopy for staging of the chronic liver disease, immunofluorescent staining for various antigens and electron microscopy for virus-like particles. Twenty-one such patients have been evaluated. Twenty have undergone percutaneous liver biopsy and eight of these have undergone an additional follow-up percutaneous liver biopsy. In the course of assaying for antigens and antibodies specific for non-A, non-B hepatitis a precipitin system has been identified that is associated with liver disease. This sytem is not specific for non-A, non-B hepatitis and is not the result of an antigen-antibody reaction. [J. H. Hoofnagle, H. J. Alter (CC), H. Denes (NIAID), G. M. Dusheiko, D. F. Schafer, G. Y. Minuk, S. M. Feinstone (NIAID), and E. A. Jones].

VII. Studies of the Uptake of Lipoproteins by Hepatic Cells

Familial high density lipoprotein (HDL) deficiency or Tangier disease is characterized by low levels of plasma HDL and apolipoproteins (apo) A-I and A-II, rapid catabolism of apo A-I and A-II and cholesterol laden reticulo-endothelial (RE) cells. Furthermore, in Tangier disease apo A-I and A-II are predominantly associated with lipoprotein particles of density less than 1.063 g/ml rather than HDL. The mechanisms underlying these abnormalities of lipoprotein metabolism in Tangier disease have been investigated. Rat endothelial, Kupffer and parenchymal cells were isolated by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. Apo A-I and A-II were isolated from normal subjects and from patients who were homozygotes for Tangier disease. After apoproteins were radioiodinated they were reassociated with lipoprotein particles of density less than 1.063 g/ml from serum of normal subjects and of patients with Tangier disease. Uptake of these labeled particles by hepatic cells *in vitro* was quantitated. The results obtained indicate that the increased RE cell uptake of cholesterol in Tangier disease is probably mediated by enhanced uptake of Tangier apo A-I by RE cells and that the rapid clearance of lipoproteins from the plasma in Tangier disease can be accounted for by parenchymal cell uptake of Tangier apo A-I. This study provides evidence that a structurally abnormal apo A-I mediates both the rapid catabolism of lipoproteins and the RE cell accumulation of cholesterol in Tangier disease. [E. A. Jones, J. A. Summerfield, J. Vergalla, E. J. Schaefer (NHLBI), and H. B. Brewer Jr. (NHLBI)].

VIII. Studies of the Uptake of Glycoproteins by Hepatic Cells

The cellular localization and carbohydrate specificities of a glycoprotein recognition system on rat hepatic sinusoidal cells have been investigated. Kupffer cells and endothelial cells were isolated by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation.

^{125}I-labeled agalactoorosomucoid (AGOR), an N-acetylglucosamine-terminated glycoprotein was selectively taken up by hepatic endothelial cells but not Kupffer cells *in vitro*. Uptake was shown to be protein dependent, calcium ion dependent and saturable and could be described by Michaelis-Menten kinetics. Uptake was inhibited not only by N-acetylglucosamine, mannose and mannan but also by glucose, fructose and a glucose-albumin conjugate. Thus, there is a glycoprotein recognition system on endothelial cells which is glucose sensitive. The existence of such a receptor suggests a mechanism for abnormal glycoprotein metabolism in diabetes mellitus. [E. A. Jones, J. A. Summerfield, J. Vergalla].

IX. Studies on the Pathogenesis of Fibrosis Following Hepatocellular Injury

Studies are being designed to explore factors involved in the development of cirrhosis. It is believed that substances are released following hepatocellular injury which cause smooth muscle cells and fibroblasts to migrate to the liver, proliferate and synthesize collagen. Chemotaxis is being assayed by measuring the degree to which cultured smooth muscle cells migrate across a pore-containing membrane in response to the presence of chemotactic factors in the culture chamber (Boyden chamber). The production of chemotactic factors by the liver of rats with carbon tetrachloride-induced hepatic fibrosis is being compared with that by the liver of normal rats. Isolated hepatic cell populations are being prepared by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. The presence of chemotactic factors is being sought in extracts of liver homogenates and supernatants of incubations of liver slices and short-term cultures of hepatic cells. Chemotactic factors will also be sought in cultures of mitogen-stimulated peripheral blood lymphocytes from patients with chronic hepatitis to test the hypothesis that in certain forms of chronic hepatitis in man, fibrosis could occur as a consequence of the sensitization of lymphocytes rather than hepatocellular injury per se. [E. A. Jones, W. G. E. Cooksley, C. McIvor, G. R. Martin (D:LDBA)].

ANNUAL REPORT SUMMARY

Digestive Diseases Branch
Phoenix Clinical Research Section

This Section conducts clinical and laboratory investigations relating to diabetes, obesity and lipoprotein metabolism primarily in Southwest American Indians. Many of the studies are done in close collaboration with the Southwestern Field Studies Section of the Epidemiology and Field Studies Branch.

We have previously shown that weight reduction improves insulin sensitivity and insulin secretory response to oral glucose in obese normal and diabetic subjects. Further studies designed to help elucidate the relationship of Type II diabetes to obesity have employed insulin therapy and exercise training. Short term (10 days) insulin therapy was shown to have no effect on sensitivity to insulin as measured by the glucose clamp technique, however, there was an improvement in the blunted insulin response to oral glucose. Six to twelve weeks of aerobic bicycle exercise resulted in improvement in maximal oxygen uptake capacity in obese normal, glucose intolerant and diabetic subjects. Exercise training resulted in improvement in insulin sensitivity in normal, glucose intolerant and mildly diabetic subjects, but no improvement was seen in moderately diabetic subjects. On the other hand, oral glucose tolerance improved in all diabetic subjects and could be attributed in part to an improvement in the early insulin response with exercise training in the diabetics.

An additional study was designed to determine if good control of plasma glucose levels in obese Type II diabetic subjects could result in a reduction of urinary protein excretion. Twenty-four hour urine samples were collected before and after hypocaloric diet therapy for a 2-4 month period in seven nondiabetic, seven glucose intolerant and twenty-seven diabetic subjects. After diet therapy, fasting and two hour plasma glucose levels after oral glucose load remained normal in nondiabetics and improved significantly in both the glucose intolerant and diabetics subjects. The concentration of plasma proteins and albumin present in the urine were measured by an immunonephelometric technique using specific antisera against human total plasma protein and albumin. No changes were found in either total protein or albumin in the nondiabetics. Protein concentrations decreased in both the intolerant and the diabetic subjects. In two cases diminished proteinuria has persisted from eight to ten months. The findings suggest that control of hyperglycemia is associated with reduction of proteinuria in Type II diabetics.

Some diabetics taking chlorpropamide have an intense facial flush after alcohol. This chlorpropamide induced alcohol flushing (CPAF) is reported to be a dominantly inherited trait associated with non-insulin dependent diabetes. CPAF has also been reported to be present before the onset of glucose intolerance and therefore recommended as a genetic marker for some cases of non-insulin dependent diabetes. The Pima Indians, a population with the highest reported prevalence of diabetes in the world have the non-insulin dependent type of diabetes. We therefore used the chlorpropamide-alcohol flushing test to study this population, to determine the relationship between CPAF and non-insulin dependent (Type II) diabetes. Studies in the Pimas have failed to show any relationship between CPAF and the presence of diabetes. Other groups have also not observed this association and therefore have failed to confirm the original observations of Leslie and Pyke.

Several groups have demonstrated that exogenous insulin inhibits endogenous insulin secretion in normal subjects. If the negative feedback constitutes an important physiological control over insulin secretion then the hyperinsulinemia of the obese would conceivably involve a defect of this regulatory system. A similar defect in regulation of insulin secretion could also partly explain the hyperinsulinemia observed in Pimas as compared to Caucasians. A study was therefore designed to examine the β cell (and α and PP cell) response to hyperinsulinemia at physiological levels of insulin (100 /ml above basal) in groups of obese and nonobese Pima Indians and Caucasians using the euglycemic clamp technique. Our studies demonstrate that C-peptide levels fall in response to hyperinsulinemia and that the effect is seen in obese as well as in lean subjects. The magnitude of the C-peptide drop was larger in the obese than in the lean, but the percentage fall was similar. During hyperinsulinemia, the suppressed C-peptide levels in the obese were still much higher than either the insulin-suppressed levels in the obese or the basal levels in the lean. Plasma glucagon levels were comparably suppressed by insulin in lean and obese subjects. The rates of recovery from insulin suppression differed in the two types of islet cells. The β cell recovered more rapidly than the alpha cells. This study presents evidence to suggest that insulin in physiological amounts can inhibit both the α and β cell and that in the obese, complete resistance of the β bell to insulin can be excluded. It is therefore possible that the hypersecretion of insulin in obese subjects could be due to (1) increased β cell mass with normal suppressibility by insulin or (2) that there is subpopulation of β cells in the islets of obese subjects which is hypersecretory and resistant to regulation by insulin.

Elevated insulin levels in Pima Indian adults compared to weight matched Caucasian controls suggest that cellular changes external to the pancreas may be important in the pathogenesis of diabetes mellitus. The aim of the cell culture project is to study several aspects of insulin function in cells obtained from normal and diabetic Pima Indians, in order to determine if diabetes mellitus is expressed in genetic differences at the cellular level. Fibroblast cultures have been established from normal and diabetic Pima Indians. Parameters of cell growth are being compared in the normal and diabetic cells, and insulin binding is being studied. In addition, cellular metabolism and transport functions are being studied in the presence and absence of insulin, in order to further investigate cell insulin action, to determine if cellular abnormalities in insulin response occur in individuals with diabetes and to investigate factors which may induce cell insulin resistance.

Regulation of insulin receptors is being studied in vivo using circulating mononuclear cells and in vitro using cultured diploid fibroblasts. Down regulation in the fibroblast has been used to detect inhibitors of insulin action in plasma from insulin resistant subjects. Investigations of monocytes and fibroblasts suggest that cells from obese subjects have more receptors, reduced insulin affinity, increased insulin degradation and decreased down regulation. Insulin binding to monocytes from obese glucose tolerant and intolerant subjects was the same, indicating that a source of insulin resistance distinct from the receptor is present in obese Pima subjects. Insulin receptors have been solubilized and partially purified from cultured human diploid fibroblasts and from human erythrocytes. Characterization of insulin binding to solubilized insulin receptor preparations resulted in binding affinity, pH optimum and analog specificity data similar to those for the intact cells. The binding of Multiplication Stimulating Activity (MSA) as well as insulin were measured in suspended human fibroblasts and in solubilized receptor preparations. Sephadex G-200 chromatography did not separate the two receptors, although characterization of the specificity of binding clearly predicts two separate binding sites.

It has been proposed that the high incidence of obesity in the Pima Indians may be due to genetic selection for a "thrifty gene", a gene that would allow the Pimas to store energy very efficiently; these efficient individuals would presumably be more capable of surviving famine than lean less-efficient individuals. In order to help determine whether or not such a genetic difference exists, thermogenic responses to various stimuli, including food, norepinephrine, and exercise, are being investigated in obese Pima volunteers. These thermogenic responses are being measured during periods of weight maintenance, of over-nutrition, and of under-nutrition. Thyroid hormone metabolism and resting metabolic rate are also being studied, since previous studies with Caucasian volunteers have shown that these parameters are affected by dietary manipulation. The results from this research are being compared to those obtained from lean and obese Caucasians involved in studies in Vermont.

Obesity in the Pima Indian is associated with a massive increase in adipose tissue mass (25-50% of total weight). The increased fat mass is due to both an increase in adipose cell size and cell number. Adipose lipoprotein lipase activity increases with obesity and is correlated with adipose cell size. Methods of studying various parameters of carbohydrate and lipid metabolism have been estabilshed using isolated human adipocytes and membrane vesicles and will be used to study human obesity.

The level of Na-pump activity has been implicated in mechanisms which could contribute to human obesity. (Na^+,K^+) ATPase activity is reduced in intact red cells and isolated erythrocyte membranes from obese Pima Indian subjects. Fibroblasts, white cells and adipocytes are being examined to determine if there is a widespread tissue loss of sodium pump activity. These cells are also being tested for a response of the Na-pump to hormones which stimulate thermogenesis. Studies of the sodium pump activity in red cells from thin and obese families and in diploid fibroblasts will be used to determine possible genetic differences in the sodium pump which relate to obesity.

The development of obesity in the Pima Indians may in part be determined by the rates of gastric emptying. Furthermore, peptic ulcer disease is known to be low in this population, but increasing. Studies of gastric emptying and gastric acid secretion are in progress and to date suggest that rates of liquid gastric emptying are not altered in obesity. Diabetes, however, appears to be associated with decreased acid secretion and hypergastrinemia and decreased gastric emptying rate. Nondiabetic Pimas have been found to have low gastric acid secretion rates and elevated gastric levels. The low acid production in the Pima Indians may explain their low incidence of gastroduodenal ulceration.

Studies of lipoprotein patterns in Indian and Caucasian diabetics and nondiabetics continue to be a major focus of investigation. Pima Indians have a high prevalence of obesity, diabetes mellitus and hyperinsulinemia, but they have low plasma cholesterol levels, reduced LDL synthesis, and decreased incidence of cardiovascular diseases. VLDL metabolism in Pima Indians is being investigated in order to further understand lipoprotein metabolism in this population. Synthesis and catabolic rates for VLDL are being compared in groups having different insulin levels using tritiated glycerol as a precursor. VLDL metabolism is being studied in a group of diabetics to determine the influence of hyperglycemia on VLDL metabolism in this population. Conversion of VLDL to LDL is being investigated in nondiabetics and diabetics using ^{125}I VLDL and ^{131}I LDL simultaneous. Postheparin, muscle and adipose tissue lipoprotein lipase levels are being measured. The results of this study should yield data on VLDL metabolism and its conversion to LDL in this population and explore the relation between these lipoproteins, diabetes and cardiovascular disease.

Both low density lipoproteins and very low density lipoproteins have been implicated as positive risk factors in the genesis of atherosclerosis, whereas high density lipoprotein concentration is an independent negative risk factor. Both diabetes and obesity are associated with changes in plasma lipoproteins and these changes may be related to the increased prevalence of cardiovascular disease. Lipoprotein composition is being examined in obese and diabetic Pima Indians. Cholesterol and triglycerides are being measured in high density lipoprotein as well as other lipoprotein subclasses isolated by ultracentrifugation. These parameters are being compared in groups of normal and diabetic Indians with and without coronary artery disease, and the influence of therapy is being assessed. Apoprotein composition of specific lipoproteins will be measured. In addition, free fatty acid levels are being studied because of their precursor relationship to lipoproteins and their reflection of insulin action. From this study we will attempt to determine if diabetes and obesity are associated with changes in serum lipoproteins and to delineate the relati

A study was undertaken to determine whether the Bio-Rad HbA$_1$ test could be used to replace the method previously used to determine glycosylated hemoglobin concentration (modifications of Gabbay and of Trivelli). Blood samples were taken from 22 normal male, 24 normal female, 11 diabetic male, and 22 diabetic female Pima Indians who were participating in epidemiological studies of diabetes on the Gila River Indian Reservation in Arizona. Both methods showed increased HbA$_1$ concentrations in the diabetic groups when methods showed increased HbA_1 concentrations in the diabetic groups when compared to the normals, but the kit values were lower, in general, than the values obtained with the previously used method. One set of control samples used revealed a need to run all assays at a constant temperature and to establish a series of frozen controls prior to beginning long-term epidemiological studies. It appears that with rigid control of pH and temperature the Bio-Rad HbA_1 test can effectively replace the more laborious method of measurement of glycosylated hemoglobin concentrations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53001-11 DDB

PERIOD COVERED
October 1, 1980 to Sept. 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies of Membrane Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Gardner	Chief, Section on Gastroenterology	DDB, NIADDK
OTHERS:	M. Jackson	Guest Worker	DDB, NIADDK
	R. T. Jensen	Senior Investigator	DDB, NIADDK
	T. Q. Garvey	Guest Worker	DDB, NIADDK
	S. M. Collins	Visiting Scientist	DDB, NIADDK
	L. Y. Korman	Clinical Associate	DDB, NIADDK
	J-P. Raufman	Clinical Associate	DDB, NIADDK
	J. P. Bali	Guest Worker	DDB, NIADDK
	M. J. Collen	Clinical Associate	DDB, NIADDK
	G. Z. Pan	Visiting Fellow	DDB, NIADDK
	M. L. Villanueva	Visiting Scientist	DDB, NIADDK
	H. Adachi	Guest Worker	DDB, NIADDK
	M. C. Beinfeld	Staff Fellow	DDB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.2	3.6	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad categories which are included in the project are: 1) Characterizing functionally the mechanism by which various substrates cross the plasma membrane of different mammalian cells; 2) identifying the metabolic and humoral factors which influence the transport of various substrates across the plasma membrane; 3) developing techniques which will distinguish between binding of a substrate to the membrane and translocation of the substrate across the membrane; 4) characterizing the mechanism by which the membrane transport of various substrates is altered in certain diseases; and 5) relating these alterations of membrane transport to the pathogenesis and clinical manifestations of the disease.

In dispersed acini from guinea pig pancreas, adding extracellular manganese causes a 4- to 5-fold increase in enzyme secretion. In addition, extracellular manganese inhibits the stimulation of enzyme secretion caused by secretagogues that mobilize cellular calcium (e.g., cholecystokinin, cholinergic agents, bombesin, physalaemin or A23187), but augments the stimulation caused by those secretagogues whose actions are mediated by cyclic AMP (e.g., vasoactive intestinal peptide or secretin). The mechanism by which manganese alters stimulated secretion differs from the mechanism by which manganese stimulates basal secretion because the change in stimulated release is maximal within 10 minutes, whereas the change in basal release does not occur until after 20 minutes. The actions of manganese on secretagogue-stimulated enzyme secretion are not attributable to manganese-induced changes in secretagogue-stimulated calcium outflux or cyclic AMP and instead, appear to result from actions of manganese on one of the later steps in the mechanism for stimulating the secretory process.

In rat pancreatic acini, secretagogues whose actions are mediated by cyclic AMP cause a 2-fold increase in enzyme secretion. Secretagogues that cause mobilization of cellular calcium produce a 7-fold augmentation of the actions of cyclic AMP-mediated secretagogues. This modulation, which is the major determinant of the magnitude of the effect of VIP and secretin on pancreatic enzyme secretion, reflects the ability of calcium mobilized from intracellular stores to amplify the action of cellular cyclic AMP on the enzyme secretory process.

In the course of testing venoms from various anthropods and reptiles for biologically active substances, we found that venom from Gila monster contains a pancreatic secretagogue. The venom augmented the increase in enzyme secretion caused by secretagogues that cause mobilization of cellular calcium but did not alter the increase in enzyme secretion caused by secretagogues whose actions are mediated by cyclic AMP. These actions result from a VIP-like substance in the venom that interacts with VIP receptors on pancreatic acinar cells to activate adenylate cyclase and increase cellular cyclic AMP. Thus, just as peptides originally isolated from frog skin (caerulein, bombesin and physalaemin) appear to represent phylogenetic precursors of chemical mediators found in mammals (CCK, gastrin-releasing peptide and substance P), the venom from Gila monster may contain a genetic ancestor of VIP.

12-0-tetradecanoyl-phorbol-13-acetate (TPA) is a potent, tumor-promoting agent extracted from croton oil. In addition to its tumor-promoting action we have found that TPA is the most efficacious pancreatic secretagogue described to date. Various other phorbol derivatives also stimulate enzyme secretion and their relative potencies for stimulating secretion are the same as their relative abilities to promote neoplastic changes. The close correlation between the relative abilities of phorbol esters to stimulate pancreatic enzyme secretion and the abilities of these agents to promote carcinogenesis suggests that these two effects may be caused by a common mechanism.

Publications:

Gardner, J. D. and Jensen, R. T.: Regulation of pancreatic enzyme secretion in vitro. In Johnson, L., Christensen, J., Grossman, M., Jacobson, E. D. and Schultz, S. G. (Ed.): Physiology of the Gastrointestinal Tract. New York, Raven Press, 1981, Vol. 2, pp. 831-871.

Gardner, J. D., Abdelmoumene, S., Lee, P. C., Collins, S. M. and Jensen, R. T.: Secretagogue-induced desensitization in pancreatic acinar cells. In Ribet, A. (Ed.): Biology of Exocrine Pancreatic Cell. Amsterdam, Elsevier/North-Holland, 1980, pp. 165-170.

Jensen, R. T. and Gardner, J. D.: Identification and characterization of receptors for secretagogues on pancreatic acinar cells. Fed. Proc. (In press).

Jensen, R. T., Collins, S. M., Pandol, S. J. and Gardner, J. D.: Lessons from studies of secretagogue receptors on pancreatic acinar cells: In Bloom, S. R. and Polak, J. M. (Ed.): Gut Hormones. Edinburgh, Churchill Livingstone, 1981, pp. 133-136.

Hyman, P. E., Brennan, M. F., Head, G. and McCarthy, D. M.: Hyperamylasemia, duodenal duplication and pleural effusions in hereditary spherocytosis. Digestive Diseases and Sciences 26: 81-84, 1981.

Abdelmoumene, S. and Gardner, J. D.: Effect of extracellular manganese on amylase release from dispersed pancreatic acini. Am. J. Physiol. (In press).

McCarthy, D. M., Nicholson, J. A. and Kim, Y. S.: Intestinal enzyme adaptation to normal diet of different composition. Am. J. Physiol. 239: G445-G451, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53002-09 DDB

PERIOD COVERED
October 1, 1980 to Sept. 30, 1981

TITLE OF PROJECT (80 characters or less)

Gastrointestinal Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Gardner	Chief, Section on Gastroenterology	DDB, NIADDK
OTHERS:	M. Jackson	Guest Worker	DDB, NIADDK
	R. T. Jensen	Senior Investigator	DDB, NIADDK
	L. Y. Korman	Clinical Associate	DDB, NIADDK
	S. Collins, N. Barlas	Visiting Scientists	DDB, NIADDK
	S. J. Pandol	Clinical Associate	DDB, NIADDK
	J-P. Bali	Guest Worker	DDB, NIADDK
	H. Adachi	Guest Worker	DDB, NIADDK
	J-P. Raufman	Clinical Associate	DDB, NIADDK
	G. Z. Pan	Visting Fellow	DDB, NIADDK
	M. Villanueva	Visiting Scientist	DDB, NIADDK
	H. Allende	Visiting Scientist	DDB, NIADDK
	B. M. Bissonnette	Clinical Associate	DDB, NIADDK

COOPERATING UNITS (if any)
Dept. of Chemistry, Case-Western Reserve Univ., Cleveland, Ohio
Div. of Cellular Biology, Kennedy Institute for Rheumatology, London, England

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.6	PROFESSIONAL: 4.0	OTHER: 1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro systems are being used to study the mechanism of action of gastrin, secretin, cholecystokinin, bombesin, substance P and vasoactive intestinal peptide with their specific membrane receptors.

Clinical investigations are directed toward developing alternative forms of therapy for and elucidating the pathogenesis of disorders characterized by ectopic production of gastrointestinal hormones (e.g., Zollinger-Ellison syndrome and pancreatic cholera).

In general, the structural requirements for occupation of a peptide hormone receptor are quite stringent in that only other peptides will bind to the hormone receptor, and the peptides that do bind are usually fragments of the hormone or analogues with a similar chemical structure. One exception to this general principle is our previous finding that butyryl derivatives of cyclic GMP are specific, selective CCK-receptor antagonists by virtue of their abilities to inhibit competitively binding of CCK to its receptors. During this past year we have discovered a second class of non-peptide CCK-receptor antagonists. Proglumide (DL-4-benzmido-N,N-dipropyl-glutaramic acid) and benzotript (N-p-chloro-benzoyl-L-tryptophan) are fully competitive antagonists of the interaction of CCK with its cell surface receptors on various target tissues. Because proglumide and benzotript are relatively inexpensive and are active following oral administration, these compounds may be useful in defining the role of CCK and related peptides in various physiological processes and may be of therapeutic value in clinical conditions in which antagonizing the actions of CCK might be expected to produce beneficial effects.

During this past year we have developed a preparation of dispersed smooth muscle cells from mammalian gastric antrum. In this preparation, gastrointestinal peptides and neurotransmitters that cause mobilization of cellular calcium promote contraction, whereas agents that increase cellular cyclic AMP cause relaxation and antagonize the contraction caused by calcium-mobilizing agents. These cells should enable us to explore in detail the cellular basis of action of agents that modify contractile activity of smooth muscle tissue.

When pancreatic acini are first incubated with CCK, washed to remove free CCK and then reincubated in fresh incubation solution, there is persistent residual stimulation of enzyme secretion. Induction of residual stimulation requires relatively high concentrations of CCK, is reversible, specific for CCK and temperature-independent. CCK-induced residual stimulation of enzyme secretion can be reversed immediately by adding a CCK-receptor antagonist such as dibutyryl cyclic GMP or proglumide.

From studies of the binding of ^{125}I-CCK and the accompanying CCK-induced changes in target cell function, we have developed a functional model for the CCK receptor. The essential features of this hypothesis are as follows: 1) Each CCK receptor has two distinct binding sites (site 1 and site 2); 2) Site 1 has a high affinity for CCK and occupation of this site is a prerequisite for occupation of site 2 by CCK; 3) The dissociation of CCK from site 1 is rapid; 4) The dissociation of CCK from site 2 is rapid when site 1 is occupied but is slow when site 1 is vacant. The pattern of the effects of CCK on its target cells depends on the states of the CCK-receptor (i.e., site 1 occupied, site 2 occupied or both sites occupied). This model not only accounts for the salient features of the actions of CCK on its target cells but predicts certain phenomena that can be examined experimentally.

Publications:

Loveridge, N., Hoile, R. W., Johnson, A. G., Gardner, J. D. and Chayen, J.: The cytochemical section-bioassay of gastrin-like activity. J. Immunoassay, 1: pp. 195-209, 1980.

Gardner, J. D., Rottman, A. J., Fink, M. L., Natarajan, S. and Bodanszky, M.: Importance of the amino acid in position 15 of VIP and secretin in influencing their interactions with membrane receptors of pancreatic acinar cells. Endocrinol. Japon., Supp. 1: pp. 1-5, 1980.

Gardner, J. D., Walker, M. D., Martinez, J., Priestley, G. P., Natarajan, S. and Bodanszky, M.: The importance of the amino acid in position 27 of cholecystokinin in determining its biological activity on pancreatic acinar cells. Biochim. Biophys. Acta 630: pp. 323-329, 1980.

Abdelmoumene, S. and Gardner, J. D.: Cholecystokinin-induced desensitization in dispersed acini from guinea pig pancreas. Am. J. Physiol. 239: G272-G279, 1980.

Collins, S. M., Abdelmoumene, S., Jensen, R. T. and Gardner, J. D.: Cholecystokinin-induced persistent stimulation of enzyme secretion in pancreatic acini. Am. J. Physiol. 240: G459-G465, 1981.

Collins, S. M., Abdelmoumene, S., Jensen, R. T. and Gardner, J. D.: Reversal of cholecystokinin-induced persistent stimulation of pancreatic enzyme secretion by dibutyryl cyclic GMP. Am. J. Physiol. 240: G466-G471, 1981.

Bodanszky, M., Tolle, J. C., Gardner, J. D., Walker, M. C. and Mutt, V.: Cholecystokinin (Pancreozymin). Synthesis and Properties of the N^{α}-Acetyl-derivative of Cholecystokinin 27-33. Int. J. Peptide Protein Res. 16: pp. 402-411, 1980.

Wunsch, E., Moroder, L., Wilschowitz, L., Gohring, W., Scharf, R. and Gardner, J. D. Zur Totalsynthese von Cholecystokinin-Pankreozymin. Hoppe-Seyler's Z. Physiol. Chem. 362: pp. 143-152, 1981.

Hahne, W. F., Jensen, R. T., Lemp, G. F. and Gardner, J. D.: Proglumide and Benzotript: Members of a New Class of Cholecystokinin Receptor Antagonists. Proc. Natl. Acad. Sci. (USA) (In press).

MITHSONIAN SCIENCE INFORMATION EXCHANGE ROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53004-09 DDB

PERIOD COVERED
October 1, 1980 to Sept. 30, 1981

TITLE OF PROJECT (80 characters or less)

Cyclic Nucleotide Mediated Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Gardner	Chief, Section on Gastroenterology	DDB, NIADDK
OTHERS:	R. T. Jensen	Senior Investigator	DDB, NIADDK
	L. Y. Korman	Clinical Associate	DDB, NIADDK
	S. Collins, N. Barlas	Visiting Scientists	DDB, NIADDK
	M. Jackson, H. Adachi	Guest Workers	DDB, NIADDK
	J-P. Bali, T. Garvey	Guest Workers	DDB, NIADDK
	M. C. Beinfeld	Staff Fellow	DDB, NIADDK
	M. L. Villanueva	Visiting Scientist	DDB, NIADDK
	J-P. Raufman	Clinical Associate	DDB, NIADDK
	G. Z. Pan	Visiting Fellow	DDB, NIADDK
	M. J. Collen	Senior Investigator	DDB, NIADDK
	H. Allende	Visiting Scientist	DDB, NIADDK
	B. M. Bissonnette	Clinical Associate	DDB, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 6.6	PROFESSIONAL: 5.0	OTHER: 1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro systems are being used to characterize the mechanism by which cyclic nucleotides alter cell function and to explore the mechanism of action of agents whose effect on cell function is mediated by cellular accumulation of cyclic nucelotides.

Previously we found that butyryl derivatives of cyclic GMP antagonize the actions of cholecystokinin (CCK) by inhibiting binding of the peptide to its membrane receptors. During the past year we have found that derivatives of cyclic AMP and cyclic IMP also inhibit binding of CCK as well as its actions on acinar cell function. The inhibition caused by cyclic nucleotide derivatives does not require the presence of a butyryl moeity because certain 8-bromo cyclic nucleotides also inhibit the interaction of CCK with its receptors. Cyclic nucleotide derivatives can also increase pancreatic enzyme secretion; however, there is no correlation between their abilities to stimulate enzyme secretion and their abilities to antagonize the actions of CCK. Finally, cyclic nucleotide derivatives also inhibit binding of ^{125}I-CCK to antibodies that are specific for the biologically active, C-terminal region of CCK. Thus, certain cyclic nucleotide derivatives possess conformational structure which resembles that of the biologically active portion of CCK and this structural similarity accounts for the abilities of these nucleotide derivatives to interact with CCK receptors and by so doing, inhibit the action of CCK in its target tissues.

ATP is the proposed chemical mediator of the so-called "purinergic nervous system". We have found that ATP, ADP and AMP, but not adenosine, are effective intestinal secretagogues. Using isolated small intestinal epithelial cells, we have found that adenine nucleotides interact with specific receptors on the outer surface of the enterocyte to cause activation of adenylate cyclase and increased cellular cyclic AMP. This same sort of action could account for the actions of adenine nucleotides on other target tissues. Thus, in intestinal enterocytes, extracellular ATP can catalyze the conversion of intracellular ATP to cyclic AMP which, in turn, produces the changes in target-cell function.

Previous studies have shown that the venous effluent from pancreatic islets passes to acinar cells before entering the general circulation. Thus, a portion of the exocrine pancreas can be exposed to relatively high concentrations of islet-cell hormones. To examine the potential significance of this anatomical connection between the endocrine and exocrine pancreas, we explored the action of glucagon on dispersed acini from guinea pig pancreas. We found that glucagon, at concentrations that would conceivably occur in the blood leaving the pancreatic islets, acts on pancreatic acinar cells to activate adenylate cyclase and increase cellular cyclic AMP which, in turn, stimulates enzyme secretion and potentiates the increase in enzyme secretion caused by secretagogues that mobilize cellular calcium.

Although streptozotocin is an effective antineoplastic agent in patients with pancreatic cholera syndrome (VIP-producing pancreatic neoplasm and profuse watery diarrhea), its use is often limited by the development of nephrotoxicity. We have found that in patients with metastatic disease to the liver, irradiation of hepatic metastases can provide a rapid and well-tolerated method of reducing tumor mass and stool volume.

Publications:

Gardner, J. D.: Pathogenesis of secretory diarrhea. In Field, M., Fordtran, J. S. and Schultz, S. (Ed.): Secretory Diarrhea. Baltimore, Maryland, Am. Physiol. Soc., 1980, pp. 153-158.

Korman, L. Y., Walker, M. D. and Gardner, J. D.: The action of theophylline on secretagogue-stimulated amylase release from dispersed pancreatic acini. Am. J. Physiol. 239: G324-G333, 1980.

Gardner, J. D. and Jensen, R. T.: Receptor modulation of calcium and cyclic AMP: Differences in coupling to specific hormone-receptor complexes in cells responsive to several hormones. Horiz. Biochem. Biophys. (In press).

Jensen, R. T.: Verner-Morrison Syndrome. Gastroenterology: A Weekly Update. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,500-08 DDB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of the Transport of Organic Anions by the Liver

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. G. Waggoner	Chemist	DDB:NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Saturable binding of sulfobromophthalein (BSP) to hepatic canalicular and plasma membranes has been demonstrated. Furthermore, proteins with high affinities for bilirubin and BSP have been isolated from hepatic cell surface membrane fractions suggesting that carrier-mediated transport mechanisms may be of importance in the hepatic uptake and excretion of these organic anions. In studies of bile salt transport it has been shown that microfilaments play a role in hepatic active transport systems.

Project Description:

Objectives:

1) To study the transport of organic anions from blood across the hepatocyte into bile, in order to characterize the biophysical nature of each of the transport steps involved in the overall hepatic excretory system.

2) To develop a capability for quantitating the function of each of these steps in patients with hepatic dysfunction by analysis of kinetic studies of various test substances such as sulfobromophthalein (BSP), indocyanine green, radiolabeled bilirubin and radiolabeled bile salts.

Methods Employed:

1) Plasma disappearance curves of labeled or unlabeled organic anions are analyzed using appropriate computer programs.

2) Pure preparations of biosynthetically labeled bilirubin conjugates are isolated from rat bile using albumin-agarose affinity columns.

3) Homogenates and cytosol of liver are subjected to thin layer chromatographic analysis of ^{3}H-labeled bile pigments after administrating unconjugated ^{3}H-bilirubin intravenously to rats. The dissociation constants of bilirubin and its conjugates for glutathione-S-transferase are measured fluorometrically.

4) Rat liver plasma and canalicular membrane fractions are prepared by differential and isopycnic centrifugation.

5) Proteins with affinities for BSP and bilirubin are isolated from rat liver membrane fractions by affinity chromatography.

6) A rapid filtration technique is used to separate cells from medium in studies of the initial uptake velocity of taurocholate by hepatocytes.

7) Polyacrylamide gel electrophoresis is used in the assessment of the effect of bile salts on protein excretion into bile.

Major Findings:

1) Fasting and postprandial serum bile acid concentrations and plasma disappearance rates of injected cholyl-glycine are entirely normal in all three subtypes of Gilbert's syndrome. These findings confirm previously fragmentary information which suggests that the transhepatic transport of bile acids occurs over separate pathways from those involved in the excretion of other cholephilic anions. They are also of clinical significance in that even minimal structural hepatic disease has been found almost invariably to be associated with an abnormality in either the postprandial serum total concentration of bile acids or plasma bile acid disappearance rates. Hence,

in patients with subtypes of Gilbert's syndrome associated with abnormalities of BSP metabolism normal bile acid studies strongly mitigates against the existence of structural liver disease and can be used as a means of obviating the necessity for invasive procedures such as liver biopsy in these patients.

2) An analysis of plasma disappearance curves of injected cholyl-glycine in normal subjects as assessed by a radioimmunoassay, which was reported by others, resulted in values for the initial volume of distribution that exceeded that of total body water and values for clearance from the plasma which exceeded hepatic blood flow. These findings prompted a further study of the disappearance of injected cholyl-glycine from plasma in normal subjects in which measurements of cholyl-glycine were made using the 3-hydroxysteroid dehydrogenase method. This study indicated that the rate of disappearance of unlabeled cholyl-glycine from plasma was the same as that of ^{14}C-labeled cholyl-glycine. This rate was similar to the corresponding rate for the more extensively studied cholephilic anion indocyanine green. Calculated values for the initial volumes of distribution of labeled or unlabeled cholylglycine appoximated to the plasma volume.

3) To obtain new insights into the relationship between plasma bilirubin turnover and hepatic bilirubin clearance, studies of bilirubin kinetics have been conducted in patients with hereditary spherocytosis before and after splenectomy. In patients whose pre-operative bilirubin clearance values were normal, the fall in bilirubin production occurring after splenectomy had no effect on hepatic bilirubin clearance, whereas in patients whose pre-operative bilirubin clearance values were low and in the range found in Gilbert's syndrome, there was a marked increase in hepatic bilirubin clearance following surgery. These findings suggest that in the patients with Gilbert's syndrome the hepatic bilirubin transport system was operating at a level close to saturation and that bilirubin loading studies may prove to be a useful provocative test for the diagnosis of carriers of the Gilbert's syndrome gene who do not express the trait at basal levels of bilirubin production.

4) The previous isolation of a pure preparation of biosynthetically labeled bilirubin conjugates in this laboratory has enabled kinetic studies of the hepatic metabolism of conjugated bilirubin to be carried out in rats. It has been shown that values for both hepatic uptake and net hepatic clearance of conjugated bilirubin are substantially faster than corresponding values for unconjugated bilirubin, presumably due, at least in part, to the ability of the former pigment to bypass the conjugation step. In addition, the hepatic uptake of conjugated bilirubin, like that of the unconjugated pigment, was inhibited by other organic anions, such as BSP and indocyanine green, but not by glycocholate, suggesting that the hepatic uptake of conjugated bilirubin is not mediated by simple diffusion but by a more complex mechanism shared with unconjugated bilirubin.

5) Bilirubin monoglucuronide accumulates preferentially within the hepatocyte whereas the diglucuronide appears to be preferentially secreted

into bile. In addition, unconjugated and conjugated bilirubins were found to have a high affinity for glutathione-S-transferases such as ligandin. Based on these findings it is suggested that microsomal monoconjugation of bilirubin may occur while it is bound to a glutathione-S-transferase and that the biliary excretion of bilirubin may involve interaction of a bilirubin monoglucuronide glutathione-S-transferase complex with the bile canalicular membrane with resultant excretion of bilirubin diglucuronide directly from the canalicular pool of the hepatocyte.

6) To assess the potential role of membrane carriers in the hepatic transport of organic anions, the kinetics of binding of labeled BSP to canalicular and plasma membrane fractions isolated from rat hepatocytes were studied. Saturable binding of BSP to both membranes was shown and this binding was competitively inhibited by bilirubin and indocyanine green but not by taurocholate. This investigation has provided further support for the hypothesis that organic anions other than bile salts share a common pathway for hepatic uptake and biliary excretion and that membrane carrier proteins may be involved in these processes.

7) Potential carrier proteins have been isolated from sinusoidal and canalicular enriched liver membrane fractions using affinity chromatography. From BSP affinity columns three binding proteins with apparent molecular weights of 4.0, 4.6 and 6.0 x 10^4 were eluted. From bilirubin affinity columns a single binding protein with an apparent molecular weight of 6.0 x 10^4 was eluted. Thus, one or more membrane carrier proteins may be involved in the hepatic transport of bilirubin and organic anionic dyes.

8) To investigate whether elevated fasting and post-prandial serum bile salt concentrations in patients with chronic liver disease might be due, at least in part, to abnormal bile salt transport, uptake of taurocholate by hepatocytes isolated from normal rats and rats with fibrosis induced by carbon tetrachloride was determined. Taurocholate uptake by hepatocytes from rats with hepatic fibrosis was noncompetitively inhibited, raising the possibility of decreased numbers of receptors for bile salts on these cells.

9) In a study of the differential effects of taurolithocholate and taurocholate on protein excretion into bile in rats, it has been shown that taurocholate induces some loss of surface proteins of the canalicular membrane without affecting the integrity of this membrane. In contrast, taurolithocholate induced substantially more protein excretion and this was associated with loss of integral canalicular membrane and Golgi components into bile. These findings may be related to the observed loss of canalicular microvilli and cholestasis caused by taurolithocholate.

10) The role of microfilaments and microtubules in bile salt transport has been studied in rats by assessing the effects of cytochalasin B, a microfilament inhibitor, and colchicine, a microtubule inhibitor, on taurocholate uptake by isolated rat hepatocytes. Cytochalasin B competitively inhibited taurocholate uptake, implying either binding of cytochalasin B to a receptor for taurocholate or direct participation of microfilaments in taurocholate uptake. Colchicine noncompetitively inhibited taurocholate uptake

suggesting either an indirect or no role of microtubules in taurocholate transport. Thus, microfilaments may be of importance in active transport systems of the hepatocyte and their functional impairment may lead to cholestasis by inhibiting bile salt transport.

11) In a further study of bile salt transport, an explanation of the anion gap in rat bile was sought. Bile salt-depleted bile was found to increase hepatocellular bile flow in a dose-dependent fashion without altering the permeability of the biliary tree. The unidentified choleretic agent was heat stable and was not a bile salt. This finding suggests that part of the bile salt independent fraction of bile may be dependent on the excretion of currently undefined anions.

Significance to Biomedical Research:

Studies of organic anion kinetics in patients with Gilbert's syndrome, Crigler-Najjar syndrome and normal volunteers have led to a marked improvement in our understanding of the excretory function of the liver. In particular, these studies have demonstrated that it is possible to quantitate hepatic function in terms of clearance values in a manner analagous to the use of creatinine clearance measurements for quantitating renal function. The ability to accurately measure both bilirubin turnover and bilirubin clearance, the two parameters which determine the plasma bilirubin concentration, has made possible a precise functional classification of all cases of unconjugated hyperbilirubinemia. Studies of the sort described above are also leading both to the development of new clinical methods for assessing the function of individual steps in organic anion transport processes and to an improved understanding of the nature of the defects in a variety of disease states which affect the liver.

Proposed Course:

This project is currently inactive. Future studies might include the following:

1) The standard protocol for defining the disappearance from plasma of intravenously administered radiolabeled unconjugated bilirubin could be extended by counting radioactivity in the upper layers of the Weber-Schalm partition of plasma samples. To the extent that the Weber-Schalm system effectively separates unconjugated from conjugated bilirubin, such data would reflect incorporation of the radiolabel into plasma conjugated bilirubin. Data of this type could be subjected to conventional curve fitting and mathematical modeling techniques.

2) Preparations of pure radiolabeled conjugated bilirubin could be used to study the kinetics of the conjugated pigment in vivo in experiments having similar designs to analogous studies that have already been conducted using radiolabeled unconjugated bilirubin.

3) A distributed modeling appraoch should be incorporated into the experimental design and analysis of future studies of bilirubin metabolism in vivo if insights into blood transport delays, short term effects within the

hepatic circulation and gradients of concentration of unconjugated and conjugated bilirubin from the portal to the centrilobular zone of the hepatic lobule are to be obtained.

Publications:

1. Reichen, J., Berk, P.D.: The role of liver plasma membrane proteins in the transport of organic anionic dyes. In: Popper, H., Bianchi, L., Gudat, F. and Reutter, W. (Eds): Communications of Liver Cells. Falk Foundation Symposium No. 27. Lancaster, England, MTP Press Ltd., 1980, pp. 59-67.

2. Reichen, J., Blitzer, B.L., Berk, P.D.: Binding of unconjugated and conjugated sulfobromophthalein to rat liver plasma membrane fractions *in vitro*. Biochim. Biophys. Acta 640: 298-312, 1981.

3. Reichen, J., Berman, M.D., Berk, P.D.: The role of microfilaments and of microtubules in taurocholate uptake by isolated rat liver cells. Biochim. Biophys. Acta 643: 126-133, 1981.

4. Jones, E.A., Carson, E.R., Berk, P.D.: The role of kinetic analysis and mathematical modeling in the study of bilirubin metabolism *in vivo*. In: Heirwegh, K. P. M. and Brown, S. B. (Eds): Bilirubin Metabolism. Boca Raton, Florida, CRC Press, 1981, in press.

5. Berk, P.D., Berman, M.D., Blitzer, B.L., Bloomer, J.R., Chretian, P., Martin, J.F., Scharschmidt, B.F., Vierling, J.M., Wolkoff, A.W., Vergalla, J., Waggoner, J.G.: Effect of splenectomy on hepatic bilirubin clearance in patients with hereditary spherocytosis: implications for the diagnosis of Gilbert's syndrome. J. Lab. Clin. Med., 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,501-08 DDB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of the Pathogenesis of Acute Hepatic Coma and the Use of Artificial Hepatic Support Devices for its Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	D. F. Schafer	Clinical Associate	DDB:NIADDK
	J. G. Waggoner	Chemist	DDB:NIADDK
	P. J. Munson	Investigator	RR:NICHHD
	M. Horowitz	Clinical Associate	POB:NCI
	P. Ferenci	Guest Worker	DDB:NIADDK
	P. Skolnick	Pharmacologist	LBC:NIADDK
	J. Fowler	Normal Volunteer	CC:NIH

COOPERATING UNITS (if any)

RR:NICHD
POB:NCI

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The abnormal pattern of visual evoked potentials in rabbits with hepatic encephalopathy (HE) due to fulminant hepatic failure (FHF) resembled that induced by barbiturates and benzodiazepines. As these drugs induce neural inhibition by interacting with receptors for the inhibitory neurotransmitter γ-aminobutyric acid on postsynaptic neural membranes, this finding implies that neuronal activity in HE is similar to that induced by GABA. Outside the CNS the main source of GABA is gut bacteria and the main site of its catabolism is the liver. When FHF was induced in rabbits the onset of HE was preceded by a marked increase in plasma GABA levels and by an increase in the permeability of the blood brain barrier (BBB). Scatchard plots of the specific binding of ^{3}H-GABA to rabbit postsynaptic membranes indicated the presence of two independent receptors for GABA. FHF was associated with a twofold increase in the number of both receptors. These findings suggest that as the liver fails: 1) impaired hepatic metabolism of GABA leads to an increase of gut-derived GABA in plasma, 2) plasma GABA gains access to the brain through a permeable BBB and 3) plasma-derived GABA may induce its own receptors and thereby increase the sensitivity of the brain to GABA.

Project Description:

Objectives:

1) It is not established whether hepatic coma is due to the accumulation of an encephalopathic substance which is inadequately cleared by the diseased liver or to a failure of the diseased liver to synthesize an adequate amount of a substance necessary for normal brain function. A major objective of this study is to obtain new insights into the pathogenesis of hepatic encephalopathy. In particular, the object is to identify and characterize potential encephalotoxins which may contribute to HE, and to determine whether they cross the blood-brain barrier. If they induce neural inhibition their site and mode of action will be investigated.

2) An additional objective is to develop extracorporeal hemoperfusion circuits for removing from plasma those metabolic products which are potentially encephalopathic and which are ordinarily extracted by the liver. In applying this approach the effects of including different adsorbents in the circuit and the effects of different hemoperfusion schedules will be assessed. An ultimate objective is the development of a biocompatible artificial liver support system for use in the management of acute potentially-reversible hepatic failure.

Methods Employed:

1) To generate an animal model of fulminant hepatic failure (FHF), the selective hepatotoxin, galactosamine-HCl, is administered intravenously according to a precise dose schedule to an inbred strain of rabbits.

2) The clearance (ml/min) of potentially encephalopathic metabolites by columns containing a variety of adsorbents has been measured using plasma supplemented with labeled and unlabeled metabolites in a simple recirculation system.

3) Rabbits in FHF have been treated by perfusing their blood over a biocompatible microporous polypropylene membrane and passing the dialysate over a column of an adsorbent before recirculating it over the membrane.

4) Visual evoked potentials in the central nervous system are measured via chronically implanted epidural electrodes. Methods have been standardized for electrode implantation and data recovery for normal rabbits and rabbits with HE due to FHF and other types of encephalopathy. Analysis of repeated recordings is performed by a dedicated computer system.

5) The concentration of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) is measured in serum and other fluids using a highly sensitive and reproducible competitive radioreceptor assay. The basis of this assay is the ability of cold GABA to displace [^{3}H]-GABA from specific receptors on preparaions of postsynaptic neural membranes isolated from brain.

6) The densities and affinities of GABA receptors on neural membranes are determined by computer assisted analysis of Scatchard plots.

7) The density and affinity of binding sites for benzodiazepines on GABA receptors are determined by analyzing Scatchard plots for the specific binding of ^{3}H-flunitrazepam to postsynaptic neural membranes.

8) The porosity of the blood-brain barrier to a radiolabeled isomer of GABA ^{14}C-α-aminoisobutyric acid is being studied using computer-assisted quantitative autoradiographic techniques.

9) The activity of the GABA-catabolizing enzyme, GABA-transaminase, is measured in tissues using a radiometric assay.

10) In studies of the metabolism of tracer doses of ^{3}H-GABA in vivo, ^{3}H-metabolites of GABA and ^{3}H-GABA itselt are separated in plasma samples by ion exchange chromatography.

Major Findings:

1) A galactosamine-induced animal model of FHF has been generated in an inbred strain of rabbit which has the advantages of being non-hazardous to personnel and reproducible. The model closely resembles human FHF in its clinical, electroencephalographic, serum biochemical and hepatic histologic features. Death occurs from liver failure. Cross circulation experiments between the model and a normal rabbit indicate that the model is potentially reversible. The rabbit is sufficiently large to permit studies of the effects of extracorporeal hemoperfusion devices on FHF.

2) Arousal of patients with HE due to FHF may be attributable, at least in part, to depletion of protein-bound neurotoxic substances from a slowly exchanging extravascular pool such as the brain or CSF. The hypothetical effects of different hemoperfusion schedules on the kinetics of depletion of a protein-bound substance, unconjugated bilirubin, have been simulated using a computer. Data on the kinetics of unconjugated bilirubin in man and experimentally determined values for the efficiency of hemoperfusion through albumin-agarose columns in removing unconjugated bilirubin from plasma were used in the simulations. The results suggest that simple alterations in the schedule of hemoperfusions might accelerate depletion of toxins from an extravascular pool and hence promote earlier arousal and possibly more frequent survival in patients with FHF.

3) Columns containing various activated carbons are ineffecient at clearing dicarboxylic amino acids and short chain fatty acids from plasma. In contrast, columns containing granular and spherical carbons are very effective at clearing from plasma aromatic amino acids and certain neurotransmitters, substances of potential importance in the pathogenesis of HE.

4) Normal rabbits, subjected to hemoperfusion schedules in which blood is perfused through silicone rubber tubing or over columns of carbon embedded in hydroxyethylcellulose, develop hyperventilation and respiratory alkalosis and subsequently die. This hemoperfusion-induced respiratory distress syndrome is associated with polymorphonuclear leukopenia and the sequestration of aggregated polymorphonuclear leukocytes in pulmonary arterioles. A heat labile factor in plasma, which is probably the activated complement component C5a, is

necessary for the development of this syndrome. Hemoperfusion through silastic tubing is not associated with this syndrome. Corticosteroids inhibit C5a-mediated aggregation of polymorphonuclear leukocytes in vitro. However, when the blood of rabbits was perfused over an implicated foreign surface, hydrocortisone 150 mg IV per kg per hour did not prevent the pulmonary sequestration of aggregated polymorphonuclear leukocytes and a fatal respiratory distress syndrome.

5) A hemoperfusion system has been devised which avoids problems of bioincompatibility due to interaction between formed elements of the blood and adsorbents, and embolization of particles of adsorbent. Blood is perfused over a microporous polypropylene membrane. The dialysate, which is in a closed circuit, is recirculated over the membrane after passage through a column of adsorbent. This arrangement avoids disadvantages associated with fluid shifts across the membrane. Normal rabbits subjected to 4-hour periods of hemoperfusion using this system have shown no ill effects.

6) The pattern and amplitude of visual evoked potentials in the central nervous system have been monitored in rabbits via chronically implanted epidural electrodes. These potentials reflect post-synaptic neuronal activity. Repeated visual stimulation with real time computer-assisted summation increased the reproducibility of visual evoked potentials by removing background electroencephalographic activity. The recording of visual evoked potentials has been shown to be a reliable, quick, non-invasive method of quantigying neurologic changes in HE. Furthermore, the pattern of visual evoked potentials associated with HE was found to be similar to that associated with coma induced by certain hypnotic agents, e.g. barbiturates and benzodiazepines, but not others, e.g. ether. As barbiturates and benzodiaepines are known to mediate their neuroinhibitory effects by interacting with postsynaptic receptors for GABA in the central nervous system, it seems possible that putative toxins which might contribute to HE might do so by interacting with the GABA inhibitory neurotransmitter system.

7) GABA metabolism and the status of GABA the neurotransmitter system have been investigated in the rabbit model of FHF. Studies involving the application of the radioreceptor assay to measure GABA and measurements of the specific binding of ^{3}H-GABA to postsynaptic neural membranes have shown: 1) A major source of GABA in peripheral blood is the enteric bacterial flora; 2) plasma levels of GABA are increased by more than an order of magnitude in FHF but not in another type of metabolic encephalopathy; (3) plasma levels of GABA in FHF become markedly elevated before the onset of HE: 4) there are two independent receptors for GABA on postsynaptic neural membranes with different binding affinities; and (5) HE is associated with no change in the affinities but with a twofold increase in the number of both receptors.

8) To determine whether GABA in plasma can gain access to the brain, the transport of the non-metabolizable isomer of GABA, α-aminoisobutyric acid (AIB), across the blood brain barrier was measured. Blood to brain transport of ^{14}C-AIB is minimal in normal rabbits but increases markedly in specific gray areas of the brain in rabbits with acute liver failure prior to the onset of HE.

9) To determine whether changes in end-organ responsivness could contribute to the increased sensitivity to hypnotic drugs, such as benzodiazepines, which

occurs in HE, the specific binding of ^{3}H-flunitrazepam to postsynaptic neural membranes of the rabbit was studied. The number of binding sites was increased in FHF, suggesting that the benzodiazepine binding site and the GABA receptor are regulated as a single operational unit and that the increased sensitivity to benzodiazepines in HE is due to the presence of more benzodiazepine receptors permitting increased drug effect.

10) The mechanism by which plasma GABA concentrations become elevated in FHF has been studied in rabbits. The activity of the enzyme which catabolizes GABA in the liver, GABA-transaminase, does not decrease in FHF. However, clearance of ^{3}H-GABA from plasma in FHF is markedly decreased, suggesting that the mechanism of the elvated plasma levels is inefficient hepatic extraction.

11) Studies of the binding of GABA to postsynaptic membranes prepared from brains obtained at human autopsies and measurements of plasma GABA levels in patients with a variety of liver and other diseases are being conducted to determine whether the findings obtained with the rabbit model of FHF apply to man. Preliminary results indicate that the number of GABA receptors is increased in the brains of patients dying of FHF or chronic hepatocellular failure but not in the brains of patients dying from a variety of other causes. Furthermore, plasma GABA levels were found to be elevated fourfold in patients with decompensated cirrhosis without overt HE but were elevated more than tenfold in patients with cirrhosis and HE or FHF.

Significance to Biomedical Research:

The mortality rate from FHF of various etiologies in man is usually 80-90%. Various types of temporary hepatic support have been used in the treatment of this syndrome in uncontrolled studies in the hope of buying time to allow hepatic regeneration to take place. Such attempts at taking over the functions of a failing liver have, to date, had little if any effect on the mortality rate. Advances in this field are impeded by continuing uncertainties regarding the pathogenesis of HE in FHF. It is not clear whether HE is due to toxic substances which impair cerebral function or to the lack of a hepatic-synthesized substance which is necessary for normal cerebral function. Furthermore, there are no reliable clinical or laboratory findings which enable the clinician to identify those patients with HE due to FHF who will die irrespective of any treatment modality, those who will recover spontaneously and those whose life might be saved by providing temporary hepatic support. Further insights into the pathogenesis of HE in FHF are being sought by studying the metabolism of inhibitory neurotransmitters and the status of receptors for inhibitory neurotransmitters in FHF. The results of the studies of the inhibitory neurotransmitter GABA in normal rabbits and rabbits in FHF suggest that as the liver fails: i) impaired hepatic metabolism of GABA leads to an increase in gut-derived GABA in plasma, ii) plasma GABA gains access to the brain through a permeable blood brain barrier and iii) plasma-derived GABA may induce its own receptors and thereby increase the sensitivity of the brain to GABA by permitting more GABA-inducing neuroinhibitory effects. This hypothesis is compatible with interactions between GABA and its receptors playing an important role in the pathogenesis of HE and with improvement in HE following partial sterilization of the gut.

The availability of an animal model of FHF enables controlled studies of the efficacy of different types of temporary hepatic support device to be conducted. The results of such studies together with a better understanding of the pathogenesis of HE should lead to more effective management of human FHF.

Proposed Course:

In the immediate future further studies of the metabolism of GABA and the status of the GABA inhibitory neurotransmitter system in HE will be conducted. These studies will include: (i) An assessment of the timing and significance of increased porosity of the blood-brain barrier in relation to the onset of HE; (ii) A determination whether a factor in plasma during liver failure is responsible for increasing the porosity of the blood brain barrier; (iii) Measurement of the binding of barbiturates and analogues of GABA, such as mucimol, to GABA receptors: (iv) Measurements of the serum level of GABA in patients with HE and other metabolic encephalopathies; and (v) Determination of the number of receptors for GABA in postsynaptic neural membranes from the brains of patients who have died from liver cell failure (acute or chronic) and other causes.

Publications:

1. Zeneroli, M.L., Schafer, D.F., Waggoner, J.G., Jones, E.A.: Hemoperfusion-induced respiratory distress syndrome in an animal model: The effects of corticosteroids. In Brunner, G. and Schmidt, F.W. (Eds.): Artificial Liver Support. New York, Springer-Verlag, 1981, pp. 189-197.

2. Schafer, D.F., Fowler, J., Jones, E.A.: Colonic bacteria: A source of γ-aminobutyric acid in blood. Proc. Soc. Exp. Biol. Med. 167:301-303, 1981.

3. Jones, E.A., Schafer, D.F.: Fulminant hepatic failure. In Zakim, D., and Boyer, T.D. (Eds.): Hepatology. Philadelphia, W. B. Saunders and Co., 1981, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,503-07 DDB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Immunologic Studies in Chronic Active Hepatitis and Primary Biliary Cirrhosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	S. P. James	Expert	MET:NCI
	W. Strober	Chief, Immunophysiology Section	MET:NCI
	C. O. Elson	Expert	MET:NCI

COOPERATING UNITS (if any)

Metabolism Branch, NCI

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	0.25

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Abnormal immune mechanisms are being studied in patients with HB_sAg negative chronic active hepatitis and primary biliary cirrhosis (PBC) selected according to strict diagnostic criteria. T cell mediated help and suppression of pokeweed mitogen-induced immunoglobulin synthesis by B cells has been studied using radioimmunoassays to measure IgG and IgM synthesized by cultures of appropriate mixtures of lymphocyte subpopulations in vitro. The ability of T cells to proliferate when cultured with either autologous or allogeneic irradiated B cells (mixed lymphocyte reactions) or with hapten-modified autologous irradiated B cells has been assessed. Results of these studies include the demonstration in PBC of (i) diminished capacity of T cells to inhibit immunoglobulin synthesis in vitro; (ii) a deficiency of the autologous but not the allogeneic mixed lymphocyte reaction and (iii) a deficiency in the primary but not the secondary proliferative response of T cells to hapten-modified cells. These findings suggest that in PBC there are defects of cell interactions involved in the generation of suppressor effector cells and recognition of exogenous antigens associated with autologous cell surface antigens.

Project Description:

Objectives:

Patients with HBsAg negative lupoid chronic active hepatitis and primary biliary cirrhosis (PBC) exhibit striking defects of immunologic function. Currently immunologic mechanisms are considered to play major roles in the pathogenesis of these two diseases. The main objective of this study is to evaluate in detail the immunologic status of patients with these two diseases. In particular, immunologic phenomena which are likely to be of pathogenic significance are studied. The ultimate goal is to identify the major pathogenic mechanisms in both diseases.

Methods Employed:

Criteria for patient selection are based on history, physical examination, results of serum biochemical tests, serum non-organ specific antibody titers and hepatic histology. Patients with a history of ingestion of hepatotoxic drugs are excluded. A few patients with alcoholic liver disease have been studied to generate control data in a chronic liver disease in which the etiology is not primarily immunologic. Similarly, a few patients with large duct biliary obstruction and sclerosing cholangitis have been studied to generate control data in patients who have cholestasis not due to PBC.

1) The immunologic status of patients is assessed by multiple intradermal antigen tests, primary immunization with keyhole limpet hemocyanin and pneumococcal polysaccharide with subsequent keyhole limpet hemocyanin skin testing, quantitation of T and B cell populations using rosetting and immunofluorescence techniques, quantitation of serum concentrations of immunoglobulins and complement components and functional (hemolytic) assays of complement components. Patients are also HLA typed.

2) For immunologic studies using peripheral blood mononuclear cells (PBMC), PBMC are harvested from blood by Ficoll-Hypaque density gradient centrifugation. T cell and non-T cell fractions are separated using Sephadex anti-$(Fab')_2$ immunoabsorbant affinity columns. PBMC are depleted of monocytes by removing cells which adhere to plastic, by removing cells which ingest iron particles by application of a magnetic field or by passing cells over Sephadex G-10 columns. Cell fractions are characterized by determining the proportions which form rosettes with sheep erythrocytes, have surface immunoglobulin detected by immunofluorescence, ingest latex beads, and are adsorbed onto a surface coated with insoluble immune complexes.

3) The cytotoxic capacity of lymphocytes and lymphocyte subpopulations harvested from peripheral blood is assessed in an in vitro cytotoxicity assay, using target cells which include Chang human hepatoma cells and mouse EL-4 sarcoma cells. The assay involves quantitating ^{51}Cr released from labeled target cells by lymphocytes from normal subjects and patients run in

parallel. The technique is used to assay spontaneous cell-mediated cytotoxicity (SCMC), mitogen induced cellular cytotoxicity (MICC) (using phytohemagglutinin, concanavalin A and pokeweed mitogen) and antibody dependent cellular cytotoxicity (ADCC) (using an appropriate anti-target cell antibody).

4) The capacity of the reticuloendothelial system to remove from the circulation particles by non-specific phagocytosis and immunospecific mechanisms is assessed. Nonspecific phagocytosis is determined from the rate of disappearance of a test dose of ^{125}I-microaggregated albumin from plasma and hepatic blood flow from the rate of disappearance of a tracer dose of this substance from plasma. Immunospecific clearance is investigated by following the fate of intravenously administered autologous red cells labeled with ^{51}Cr and coated with a pure IgM or IgG antibody directed against ABO or Rh antigens, respectively. Clearance of IgM coated cells occurs largely in the liver and depends on the interaction between C3b deposited in vivo on the red cell membrane and specific C3b receptors on Kupffer cells, while clearance of IgG coated cells is mediated by Fc-receptor-bearing fixed macrophages in the spleen. After clearance IgG-coated cells undergo phagocytosis, whereas IgM-coated cells either undergo phagocytosis or release back into the circulation as C3d-coated cells as a result of the action of circulating C3b inactivator.

5) The regulation of immunoglobulin synthesis by B cells is studied by quantitating T cell mediated help and suppression of pokeweed mitogen-induced immunoglobulin synthesis in vitro. This is achieved by culturing B cells with unirradiated or irradiated T cells at different T:B cell ratios and measuring IgG and IgM in culture supernatants using sensitive radioimmunoassays. Immunoglobulin synthesis by autologous and allogeneic mixtures of T and B cells from patients and control subjects are studied.

6) The ability of T cells to proliferate when cultured with either autologous or allogeneic irradiated non-T cells is determined. These phenonema are known as the autologous and allogeneic mixed lymphocyte reactions (MLR), respectively. Incorporation of ^{3}H-thymidine into T cells is used as an index of cell proliferation.

7) The surface of peripheral blood mononuclear cells and B cells are hapten-modified by treating the cells with trinitrobenzenesulfonic acid.

Major Findings:

1) There is a higher incidence of skin test anergy in patients with PBC than in patients with chronic active hepatitis and controls. This anergy includes the response to de novo immunization with keyhole limpet hemocyanin.

2) The cytotoxic response in patients with PBC and HBsAg negative chronic hepatitis is not specific for the Chang cell and does not indicate prior sensitization in vivo to antigens on this target cell.

3) In contrast to chronic hepatitis, SCMC of lymphocytes is defective in PBC. There is no defect in MICC or ADCC in either disease. In both diseases SCMC and ADCC are mediated by a subpopulation of lymphocytes which lack surface immunoglobulin and bear Fc receptors, whereas MICC is mediated

by lymphocytes which lack both surface immunoglobulin and Fc receptors. Thus, there is a difference in cytotoxic function between PBC and chronic hepatitis in that the former but not the latter disease is associated with a defect in the spontaneous cytotoxic function of lymphocytes which lack surface immunoglobulin but bear Fc receptors.

4) Starting with peripheral blood mononuclear cells, depletion of complement receptor-bearing cells is associated with enrichment of Fc positive and surface immunoglobulin negative cells, depletion of phagocytes and surface immunoglobulin positive cells and normal or enhanced SCMC, MICC and ADCC. In contrast, depletion of Fc positive cells is associated with enrichment of complement receptor-bearing cells and virtually no capacity to induce SCMC or ADCC. These findings indicate that at least the majority of cells which mediate SCMC, MICC and ADCC do not bear complement receptors.

5) The clearance of both tracer and test doses of microaggregated albumin is normal in patients with compensated PBC, alcoholic cirrhosis and HBsAg negative chronic hepatitis, indicating normal hepatic blood flow and a normal non-specific phagocytic capacity of the reticuloendothelial system. The rate of clearance of IgG-coated radiochromated autologous red cells is normal in patients with compensated HBsAg negative chronic hepatitis and alcoholic cirrhosis. However, in the majority of patients with PBC, the rate of clearance of these cells was increased. This observation may imply activation of the reticuloendothelial system in this disease. In contrast, the clearance of C3b-coated radiochromated autologous red cells is markedly decreased in patients with compensated PBC, but is normal in patients with compensated HBsAg negative chronic hepatitis, alcoholic cirrhosis and large duct biliary obstruction. To obtain further insight into this clearance defect in PBC, a compartmental model of the C3b receptor-mediated clearance process has been developed. Using computer simulations the model is able to account for the observed data and shows that the major determinant of the defect is a decreased rate of deposition of C3b-coated cells on Kupffer cell receptors. The defect cannot be attributed to cholestasis, to deficiency of components of the classical complement pathway, or to increased activity of C3b-inactivator. However, the defect may be due to a decrease in the number or affinity of C3b-receptors or to partial saturation of these receptors by excess circulating C3b or immune complexes containing C3b.

6) Cultures containing unirradiated T cells and B cells from normal individuals show augmentation of immunoglobulin (Ig) synthesis at low T:B cell ratios (helper T cell effect) and inhibition of Ig synthesis at high T:B cell ratios (suppressor T cell effect). Cultures containing irradiated T cells from normal individuals show augmentation of Ig synthesis at all T:B cell ratios studied, indicating marked reduction of suppressor T cell function by irradiation. In contrast, cultures containing unirradiated T cells and autologous B cells from patients with PBC do not show inhibition of Ig synthesis at high T:B cell ratios, but instead show progressive increases in Ig synthesis with increasing T:B cell ratios up to the highest T:B cell ratio studied (10:1). Ig synthesis by cultures containing irradiated T cells and autologus B cells from patients with PBC is similar to that by corresponding cultures of cells from normal subjects. Allogeneic mixtures of cells from

normal individuals and patients with PBC show that, while B cells from the majority of patients with PBC synthesize normal amounts of Ig in the presence of normal T cells, T cells from patients with PBC cause marked enhancement of Ig synthesis by normal B cells. Patients with other liver diseases characterized by chronic cholestasis, cirrhosis, or hepatocellular necrosis do not exhibit an analogous abnormality of Ig synthesis *in vitro* to that found in PBC. Thus, PBC is associated with a defect of immune regulation characterized by abnormally high Ig synthesis by cultures of autologous T and B cells at high T:B cell ratios. This defect appears to be due to a deficiency of suppressor T cell function.

7) Patients with PBC have a marked deficiency in the ability to generate the autologous mixed lymphocyte reaction but have a normal ability to generate an allogeneic mixed lymphocyte reaction. The defect in the autologous mixed lymphocyte reaction is not due to a serum factor. The deficiencies of both suppressor T cell function and the autologous mixed lymphocyte reaction in PBC could be due to a common defect of cell interactions involved in the generation of suppressor effector cells. Such a defect could play an important role in the pathogenesis of autoimmune disease.

8) The proliferative responses of peripheral blood mononuclear cells (PBMC) and T cells when cultured with hapten-modified irradiated stimulator cells have been determined. The stimulator cells were autolous PBMC or B cell-enriched PBMC. The results indicated that the primary but not the secondary proliferative response is decreased in PBC. This finding suggests that in PBC there is a decreased proliferative response of a subpopulation of suppressor T cells which may be responsible for a decreased recognition of exogenous antigens in association with autologous cell surface antigens.

Significance to Biomedical Research:

Elucidation of the pathogenic mechanisms which initiate and sustain the disease processes of chronic active hepatitis and PBC is required for the development of more rational therapies. Defined initiating factors exist for those subgroups of chronic active hepatitis which are associated with HBsAg and which follow the ingestion of certain drugs, whereas initiating factors are unknown for most of the remainder of patients with chronic active hepatitis, (i.e. patients with lupoid hepatitis) and for all patients with PBC. The processes leading to sustained disease activity are unknown in both conditions. Current hypotheses favor immunologic mechanism(s). These hypotheses, in part, stem from the high prevalence of abnormal immunologic phenomena in both diseases. The empiric use of adrenal corticosteroids and other immunosuppressive therapies in these diseases appears to be, at least in part, a reflection of this view. To date, existing specific hypotheses relating to the pathogenesis of these diseases lack experimental confirmation. In addition, it is not known whether certain immunologic abnormalities found in some subgroups of patients and not in others are epiphenomena or are related to pathogenesis.

The current studies are designed to provide a comprehensive immunologic evaluation of selected patients so that the spectrum of immunologic abnormalities associated with PBC and HBsAg negative lupoid chronic hepatitis can be defined and the relationship of these abnormalities to clinical and histologic variables can be assessed. In addition, hypotheses of specific pathogenic mechanisms are tested. The significant morbidity and mortality of chronic active hepatitis, the apparently uniform mortality of symptomatic PBC, and the inadequacies of all current therapies in both conditions emphasize the need for information which throws light on the pathogenesis of these diseases.

Proposed Course:

Modern sophisticated immunologic techniques will be applied to define in greater depth the nature and significance of the immunologic defects exhibited by patients with HBsAg negative chronic active (lupoid) hepatitis and PBC. Particular emphasis is being placed on investigating siblings of patients with PBC to assess the importance of hereditary traits in this disease and to facilitate studies requiring the mixing of HLA identical cells from normal subjects and patients with PBC.

Publications:

1. Jones, E.A., James, S.P., Vierling, J.M.: Immunologic function in primary biliary cirrhosis: Defects recently demonstrated at the National Institutes of Health. In Chalmers, T.C. and Berk, P.D. (Eds.): Frontiers in Liver Diseases. New York, Stratton Intercontinental, 1981, pp. 283-304.

2. James, S.P., Elson, C.O., Waggoner, J.G., Jones, E.A., Strober, S.: Deficiency of the autologous mixed lymphocyte reaction in patients with primary biliary cirrhosis. J. Clin. Invest. 66: 1305-1310, 1981.

3. Jones, E.A., Vierling, J.M., Steer, C.J.: Surface receptors on hepatic sinusoidal cells. In Popper, H., Bianchi, L., Gudat, F., Reuter, W. (Eds.): Communications of Liver Cells. Falk Symposium No. 27, Lancaster, England, MTP Press Ltd., 1980, pp. 211-227.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 53,504-07 DDB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Copper Metabolism in Man Using ^{67}Cu and ^{64}Cu

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	R. Aamodt	Senior Radiobiologist	NM:CC
	W. Rumble	Biologist/Radiobiologist	NM:CC
	R. Shrager	Mathematician	LSMM:CR

COOPERATING UNITS (if any)

Nuclear Medicine Department, CC
Laboratory of Statistical and Mathematical Methodology, CR

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

X (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is currently inactive.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 53,505-06 DDB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies of the Metabolism of Alpha-1-Antitrypsin and of Protease Inhibitor Phenotypes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. Vergalla	Chemist	DDB:NIADDK

COOPERATING UNITS (if any)
Pulmonary Branch, NHLBI

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Isolated preparations of α-1-antitrypsin (α1AT), trace-labeled with radioiodine, have been used to obtain data on the turnover of α1AT in normal subjects and patients with α1AT deficiency who have either pulmonary emphysema or chronic hepatocellular disease. The protease inhibitor (Pi) phenotypes of subjects donating plasma for isolation of α1AT and subjects undergoing α1AT turnover studies have been determined. The fractional catabolic rates of M α1AT from normal subjects (phenotype PiMM) and Z α1AT from patients with α1AT deficiency (phenotype PiZZ) are similar in both normal subjects and patients with α1AT deficiency. These findings imply that the low serum α1AT concentration in α1AT deficiency is due to impaired hepatic release of α1AT and that the differences in molecular structure between the Z and M proteins have little effect on the catabolism of α1AT. In common with other glycoproteins, desialylated (i.e. neuraminidase-treated) α1AT is rapidly cleared from plasma by the liver. Pi phenotypes have been determined in populations of patients with rheumatoid arthritis, systemic lupus erythematosis, Sjogren's syndrome and hepatocellular carcinoma.

Project Description:

Objectives:

Alpha-1-antitrypsin (α1AT) is a plasma glycoprotein which contains sialic acid and is responsible for most of the tryptic inhibitory capacity of plasma. The objectives of this study are to obtain data on the metabolism of α1AT in normal subjects (protease inhibitor [Pi] phenotype MM), patients with α1AT deficiency (PiZZ), relatives of PiZZ individuals with one Z gene and patients with hepatocellular disease unassociated with α1AT deficiency. The possibility that the degree of sialylation of this protein, which is less in PiZZ than PiMM individuals, influences its metabolism _in vivo_ will be investigated by comparing the metabolism of α1AT's isolated from PiMM and PiZZ individuals in the same subjects and by studying the metabolism of α1AT which has been desialylated _in vitro_. The necessity to perform Pi phenotyping for these studies permits additional studies in which the distribution of Pi phenotypes in selected populations is determined.

Methods Employed:

1) Pi phenotypes are determined by acid starch gel electrophoresis followed by crossed immunoelectrophoresis using a specific anti-serum to α1AT and by slab gel isoelectric focusing in acrylamide.

2) α1AT is isolated from plasma using $(NH_4)_2SO_4$ precipitation, gel exclusion chromatography, Concanavalin A affinity chromatography, DEAE ion exchange chromatography and affinity chromatography using blue dextran and activated thiol exchange.

3) The purity of isolated α1AT is determined using crossed immunoelectrophoresis, polyacrylamide gel electrophoresis and isoelectric focusing. Its biological activity is assessed by a kinetic assay of tryptic inhibitory capacity using an artificial substrate.

4) To quantitate the turnover of α1AT in man, pure biologically active α1AT trace labeled with radioiodine is administered intravenously and serial measurements of radioactivity in the whole body, plasma and urine are analyzed.

Major Findings:

1) Isolated (PiMM) α1AT has been shown to be immunochemically pure and to retain both its characteristic electrophoretic microheterogeneity on acid starch gel electrophoresis and its ability to inhibit trypsin.

2) The half life of radioiodinated human (PiMM) α1AT in the plasma of rabbits varied from 8.7 to 10.4 hours whereas that of the same protein after desialylation was less than 10 minutes.

3) Ranges for pool sizes, transfer rates between pools and synthetic and fractional catabolic rates of α1AT in normal subjects have been determined.

4) In turnover studies of MM α1AT in normal (PiMM) volunteers, the serum concentration of α1AT was shown to vary independently of its fractional catabolic rate suggesting that a constant volume of plasma is cleared of α_1AT per unit time. Analysis of the turnover data indicated that α_1AT is catabolized in, or in close relationship to, the intravascular compartment.

5) In analogous studies in normal volunteers the desialylated protein was shown to be removed from plasma approximately 150 times faster than the intact protein. Its rapid disappearance from plasma was associated with an equally rapid uptake of the radioiodine label by the liver. This finding emphasizes that the catabolism of α1AT can be influenced by the molecular structure of the carbohydrate moiety and suggests that hepatic receptors for desialylated glycoproteins exist in the human liver.

6) A patient with the rare Pi phenotype SZ, α1AT deficiency and cirrhosis associated with globules of α1AT material in his hepatocytes was shown to have appreciable hepatic copper overload, chronic fibrosing interstitial pneumonitis and, terminally, a hepatocellular carcinoma.

7) The fractional catabolic rate of the Z protein in patients with α1AT deficiency is similar to that of the M protein in normal subjects. Furthermore, the fractional catabolic rates of the M protein in patients with α1AT deficiency and of the Z protein in normal subjects are similar to that of the M protein in normal subjects. Thus, the low serum concentration of α1AT in α1AT deficiency is due to impaired hepatic release of α1AT rather than hypercatabolism of α1AT. Futhermore, differences in the molecular structure of the Z and M proteins do not affect the catabolism of α1AT to any appreciable extent.

8) No significant increase in non-MM Pi phenotypes was found in 37 patients with rheumatoid arthritis and 40 patients with systemic lupus erythematosus suggesting that particular Pi types neither predispose to the development nor enhance the severity of these diseases.

9) No significant increase in non-MM Pi phenotypes was found in 52 patients with Sjogrens syndrome. Mean values for tests of pulmonary function, including estimates of both restrictive lung disease and airway obstruction were not significantly different for patients with Sjogren's syndrome who had the Pi MM phenotype and those with non-MM Pi phenotypes. Thus, patients with Sjogren's syndrome who have non-MM Pi phenotypes (partial α1AT deficiency) do not appear to have an increased susceptibility to develop chronic obstructive pulmonary disease.

Significance to Biomedical Research:

Homozygous α1AT deficiency (phenotype PiZZ) may be associated with cholestasis in the neonatal period, cirrhosis, liver cell carcinoma and emphysema at a young age. In this condition, hepatocytes have been shown to contain globules of α1AT-like material which is devoid of sialic acid, and the α1AT in plasma, which is present in abnormally low concentration, has also been shown to have a reduced content of sialic acid. The relationship of the characteristic hepatic globules and the low plasma concentration of α1AT to the hepatic lesions associated with α1AT deficiency is unknown. This study is designed to investigate the metabolism of α1AT in α1AT deficiency. The relationship of the characteristic hepatic globules to the rate at which α1AT is delivered to plasma and to the concentration of α1AT in plasma is assessed. In addition, the contribution of increased catabolism or hepatic uptake of α1AT to reduced plasma concentrations of α1AT is determined. The study determines the effect of certain differences in the molecular structure of α1AT (e.g. different degrees of sialylation) on its rate of endogenous catabolism and hepatic uptake. In particular, the hypothesis that there are hepatic receptors for exposed galactose residues on desialylated glycoproteins which facilitate the rapid hepatic clearance of these proteins is tested in man.

The observation that desialylated glycoproteins appear to be metabolized by hepatic lysosomes suggests that measurements of hepatic radioactivity after administering a radioiodinated desialylated glycoprotein such as α1AT could be used as a test for hepatic lysosomal function.

The determination of Pi phenotypes enables the frequency distribution of this genetic marker to be determined in well-defined populations of patients.

Proposed Course:

Pi phenotypes of sera from a large number of black South African patients with hepatocellular carcinoma and from age and sex matched healthy black South Africans are being determined to assess whether the incidence of any particular Pi phenotypes is increased in the patient population.

Publications:

1. Karsh, J., Moutsopoulos, H. M., Vergalla, J., Jones, E. A.: Protease inhibitor phenotypes and pulmonary disease in patients with Sjogren's syndrome. Respiration 41: 60-65, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,506-05 DDB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of the Properties of Desialylated Human Lymphocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	C. J. Steer	Expert	LBM:NIADDK
	S. P. James	Expert	MET:NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Desialylated (i.e. neuraminidase-treated) lymphocytes (DL) induce significantly more cytotoxicity in isolated rabbit hepatocytes than do intact lymphocytes. This phenomenon can be selectively blocked by a desialylated glycoprotein and is attributed to the receptor for desialylated glycoproteins (hepatic binding protein, HBP) on the surface of hepatocytes interacting with its specific ligands on the surface of DL. Purified HBP increases the cytotoxicity of DL to target cells which do not bear HBP on their surface presumably by an analogous mechanism. Infused desialylated periperal blood mononuclear cells (PBMC) and DL are selectively taken up by the liver in rabbits. The phenomenon is unaffected by depletion of complement but can be blocked by preincubation of desialylated PBMC with IgG-Fab fragments. These findings suggest that the selective hepatic uptake of desialylated PBMC or DL is not mediated by HBP. They are compatible with desialylated PBMC or DL becoming coated with naturally occurring antibodies in vivo with the result that subsequent recognition of cell bound antibody by Fc receptors on Kupffer cells mediates their selective hepatic uptake.

Project Description:

Objectives:

Hepatic binding protein (HBP), a normal constituent of the surface membrane of mammalian hepatocytes, can induce mitogenesis in desialylated lymphocytes. This project is designed to determine whether HBP has additional immunologic properties such as the ability to induce desialylated lymphocytes to become cytotoxic, that is to mediate mitogen-induced cellular cytotoxicity (MICC). Desialylated lymphocytes are selectively taken up by the liver. Studies are designed to determine the probable mechanism of this phenomenon, including the role, if any, of HBP.

Methods Employed:

HBP is isolated in a pure form from rabbit liver using affinity chromatography. HBP in the surface membranes of hepatocytes is measured by its ability to bind ^{125}I-asialo-orosomucoid. For assays of cytotoxicity lymphocytes are isolated from the blood of normal human volunteers by centrifugation over Ficoll-Hypaque. Lymphocytes are desialylated by incubating with neuraminidase. Two in vitro assays of cytotoxicity are used. One measures the release of radioactivity from ^{51}Cr-labeled nucleated target cells after their incubation with effector cells in the presence or absence of mitogens. The other measures the loss of ability of target cells to adhere to plastic after incubation with effector cells. Viability of both effector and target cells is measured by the ability of cells to exclude the dye trypan blue. Chang cells are used as nonspecific nucleated target cells. Pure rabbit hepatocytes, isolated by perfusing the liver in situ with a solution containing collagenase and hyaluronidase, are used as hepatospecific target cells containing exposed HBP on their surface membrane.

After the infusion of ^{51}Cr-labeled desialylated and normal peripheral blood mononuclear cells (PBMC) and lymphocytes into rabbits the fate of labeled cells is determined from measurements of radioactivity in plasma, liver and other organs. The effects of complement depletion using Cobra venom factor and preincubation with IgG-Fab fragments on the hepatic uptake of labeled desialylated PBMC are quantitated.

Major Findings:

1. Cytotoxicity of desialylated lymphocytes against nonspecific nucleated target cells in the presence of HBP is equivalent to that in the presence of the powerful plant mitogen phytohemagglutinin and is much greater than that induced by these cells in the absence of HBP. Having shown that isolated rabbit hepatocytes contain exposed HBP in their surface membrane, it was found that a much greater proportion of these cells lose their ability to adhere to plastic after incubation with desialylated lymphocytes than after incubation with normal untreated lymphocytes. This effect of desialylated lymphocytes was specifically inhibited by asialo-orosomucoid, a desialylated glycoprotein, but not by intact orosomucoid. These findings indicate that HBP can mediate MICC not only as a purified protein but also as a constituent of the surface membrane of a mammalian hepatocyte.

2. The selective hepatic uptake of the label of ^{51}Cr-labeled desialylated peripheral blood mononuclear cells (PBMC) and desialylated lymphocytes has been demonstrated in the rabbit. This phenonenon does not appear to be due to the specific interaction between exposed galactose residues on the surface of the desialylated cells and HBP which specifically recognizes exposed galactose residues on desialylated glycoproteins, since sinusoidal cells, which come into contact with circulating desialylated cells do not bind desialylated glycoproteins and hence do not bear HBP. Consistent with this reasoning is the observation that after infusions of desialylated lymphocytes into rabbits neither serial values for serum transaminases nor hepatic histology were indicative of hepatocellular injury. Naturally occurring antibodies exist in normal plasma which specifically recognize antigens exposed on the surface of lymphocytes by desialylation. These antibodies probably do not mediate the selective hepatic uptake of desialylated cells through the action of complement as complement depletion of rabbits in vivo was without effect on the hepatic uptake of the label of ^{51}Cr-labeled desialylated cells. However, preincubation of labeled desialylated PBMC with rabbit IgG Fab fragments reduced the hepatic uptake of the label of these cells to that of the label of ^{51}Cr-labeled intact PBMC, suggesting that the Fab fragments were able to bind to exposed antigens on desialylated cells and inhibit binding of circulating antibodies to these antigens. These findings are compatible with the hypothesis that desialylated PBMC or lymphocytes in vivo become coated with naturally occurring antibodies with subsequent recognition of cell-bound antibody by Fc receptors on Kupffer cells mediating their selective hepatic uptake.

Significance to Biomedical Research:

For the first time MICC has been shown to be mediated by a normal constituent of the surface membrane of a mammalian cell. The results of these studies demonstrate that a minor structural change in the surface membrane of lymphocytes might result in their becoming cytopathogenic when exposed to normal hepatocytes. Thus MICC may be a mechanism of hepatocellular injury in vivo. This observation raises the possibility that interaction between a variety of constituents of normal cell membranes and lymphocytes whose surface membranes have undergone subtle structural changes may be a mechanism of immune-mediated cellular injury in human disease.

The clearance of desialylated lymphocytes by Fc receptors on Kupffer cells in vivo may prevent the mediation of hepatocellular injury by the interaction of desialylated lymphocytes with HBP on the surface of hepatocytes. This may be particularly significant since the enzyme responsible for desialylation, neuraminidase, appears to be widely distributed in vivo and occurs in organisms pathogenic to man such as the influenza virus. The coating of desialylated cells by naturally occurring antibodies in vivo and their subsequent accelerated clearance from the circulation by Fc receptors on Kupffer cells may be a general phenomenon which applies to all formed elements of the blood including platelets. For instance, it may be a physiologic mechanism for the removal of senescent red cells from the circulation as the surface membrane of such cells contains much less sialic acid than the surface membrane of young red cells.

Proposed Course:

No further studies are planned.

Publications:

1. Steer, C.J., James, S.P., Vierling, J.M., Hosea, S.W., Jones, E.A.: The selective hepatic uptake of desialylated peripheral blood mononuclear cells in rabbits. Gastroenterology 79: 917-923, 1980.

2. Jones, E.A., Summerfield, J.A.: Kupffer cells. In Arias, M., Popper, H., Shafritz, D., Schacter, D., Jakoby, W. (Eds.): The Liver: Biology and Pathobiology. New York, Raven Press, 1981 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,507-04 DDB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Synthesis and Binding of Collagen by Hepatocytes in Chronic Liver Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 53,508-04 DDB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Hepatic Receptors for Modified Glycoproteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. A. Summerfield	Visiting Scientist	DDB:NIADDK
	G. Ashwell	Chief, Lab. Biochem. and Metab.	LBM:NIADDK
	J. Vergalla	Chemist	DDB:NIADDK

COOPERATING UNITS (if any)

Laboratory of Biochemistry and Metabolism

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2 .25	1.25	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cellular location and carbohydrate specificies of a glycoprotein recognition system on rat hepatic sinusoidal cells have been determined. Purified preparatons of endothelial, Kupffer and parenchymal cells have been prepared by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. ^{125}I-labeled agalactoorosomucoid (AGOR), an N-acetylglucosamine-termined glycoprotein, is selectively and specifically taken up in vitro by endothelial cells. Glucose competitively inhibited this uptake process over a wide range of concentrations. Uptake by cells from fasted rats was enhanced, but uptake by cells from fasted or fed diabetic rats was normal. The in vivo, hepatic uptake and catabolism of ^{125}I-AGOR were slower in diabetic than normal rats. These findings indicate that 1) the hepatic receptors which recognize N-acetylglucosamine/mannose terminated glycoproteins are located on endothelial cells, 2) these receptors are glucose sensitive, 3) fasting increases the number of these receptors and 4) diabetes mellitus abolishes this effect of fasting and impairs the function of this receptor in vivo.

Project Description:

Objectives:

This study was designed to determine whether receptors for modified glycoproteins, other than the receptor previously described for galactose-terminated glycoproteins (HBP), exist in the mammalian liver. It was also designed to assess the distribution of receptors for modified glycoproteins, including HBP, between parenchymal and non-parenchymal hepatic cells. The potential implications of these receptors to clinical medicine have been the subject of additional studies. In particular, the effects of modifying the carbohydrate moiety of glucocerebrosidase, the enzyme deficient in Gaucher's disease, on its cellular distribution within the liver after infusion have been determined. The cell type responsible for the rapid hepatic clearance of a lysosomal enzyme (β-hexosamindase A) and the glycosyl specificity of this process has also been investigated.

Using isolated hepatocytes the intracellular:cell surface membrane distribution ratio of HBP, the stability of HBP and the ability of HBP to recycle between the cell membrane and intracellular structures have been studied.

The cellular location and carbohydrate specificies of hepatic receptors which recognize N-acetylglucosamine and mannose-terminated glycoproteins have been determined.

Methods Employed:

1) Suspensions of hepatic cells are obtained from rat liver after in situ perfusion with a solution of collagenase. Parenchymal cells (hepatocytes) are subsequently isolated from cell suspensions by centrifugation on Percoll gradients. Kupffer cells and endothelial cells are separated by centrifugal elutriation.

2) For binding studies, the carbohydrate moiety of native glycoproteins is modified by incubations with appropriate enzymes (neuraminidase, β-galactosidase, N-acetylglucosaminidase).

3) Glucocerebrosidase activity is assayed in detergent buffered extracts of sonicated cells.

4) Glycoproteins are radioiodinated using a solid phase lactoperoxidase glucose oxidase system.

5) Standard assay techniques are employed to determine the uptake of labeled ligands by cells incubated in vitro. The amount of unlabeled ligand mixed with the appropriate labeled ligand is varied. Catabolism of labeled ligands is assessed by measuring the accumulation of acid soluble radioactivity in the incubation medium.

6) To study the kinetics of binding of ^{125}I-labeled galactose terminated glycoproteins to HBP, isolated hepatocytes are maintained in suspension.

Cycloheximide is used to inhibit protein synthesis by such cells. Cells are subjected to detergent solubilization to determine the total amount of HBP in hepatocytes (intracellular and surface membrane).

7) Isolated cells are characterized by staining with specific histochemical stains, measuring the activities of specific cell enzymes and assessing the presence of cell surface receptors for C3b and IgG-Fc by determining the ability of cells to form rosettes with sheep erythrocytes coated with IgG or IgM and complement.

8) Flow cytometry is performed in a cell sorter.

9) Studies of the metabolism of labeled ligands in vivo are conducted using a standard protocol for determining the turnover and fate of a tracer dose of labeled ligand administered by intravenous injection.

Major Findings:

1) Significant binding of galactose, but neither N-acetylglucosamine nor mannose-terminated orosomucoid to intact hepatocytes was demonstrated. Non-parenchymal cells did not bind galactose-terminated orosomucoid but bound significant amounts of N-acetylglucosamine and mannose-terminated orosomucoid. Thus, Kupffer cells lack a receptor for galactose-terminated glycoproteins. Carrier dilution studies indicated the presence of a distinct receptor for galactose-terminated glycoproteins on hepatocytes and a distinct receptor with mannose specificity on non-parenchymal cells.

2) The carbohydrate moiety of the enzyme glucocerebrosidase can be modified without the glycoprotein losing enzyme activity. Galactose-terminated glucocerebrosidase is preferentially taken up by hepatocytes. Exposure of N-acetylglucosamine or mannose sugar residues markedly augments the uptake of glucocerebrosidase by non-parenchymal hepatic cells.

3) The rapid in vivo hepatic uptake of the glycoprotein β-hexosaminidase A exhibits mannose specificity. In vitro no uptake of this enzyme by hepatocytes is detectable, whereas there is appreciable uptake of the enzyme by sinusoidal cells. This uptake process also exhibits mannose specificity. The kinetics of uptake of β-hexosaminidase A and mannose-terminated orosomucoid by sinusoidal cells are similar.

4) Of the total HBP in rat hepatocytes 5% is on the cell surface and 95% is intracellular. The average time an individual HBP receptor remains on the cell surface is 3 minutes. More ^{125}I-labeled galactose-terminated glycoprotein is metabolized in hepatocytes per unit time than can be bound by the total number of intracellular and surface membrane HBP receptors under conditions of inhibition of protein synthesis. This finding indicates that HBP exhibits no down regulation and suggests that the receptor is either recycled or is re-utilized at its plasma membrane site.

5) Cells in Kupffer cell preparations have a larger modal cell volume and protein content per cell than cells in endothelial cell preparations. A much higher proportion of Kupffer cells than endothelial cells are in a proliferative phase of the cell cycle. Large proportions of cells in Kupffer cell fractions

stain positively for endogenous peroxidase and tartrate resistant acid phosphatase and possess surface receptors for C3b and IgG-Fc.

6) Fasting prior to isolation of cells decreases the recovery of endothelial cells by 60% and of Kupffer cells by 32%. Kupffer cells isolated from fasted animals have a larger modal cell volume than those isolated from fed animals. Diabetes mellitus reduces the recovery of endothelial cells by 50%.

7) ^{125}I-labeled agalactoorosomucoid (AGOR), an N-acetylglucosamine terminated glycoprotein is selectively taken up by endothelial cells. This process is protein dependent, calcium ion dependent, saturable and can be described by Michaelis-Menton kinetics. It is inhibited by N-acetylglucosamine, mannose, mannan, glucose, fructose and a glucose-albumin conjugate.

8) Fasting of normal rats increases the number of receptors for AGOR on isolated endothelial cells. This effect of fasting was abolished by inducing diabetes mellitus.

9) The plasma disappearance rate, hepatic uptake and cataboslim of ^{125}I-AGOR are slower in diabetics than normal rats.

Significance to Biomedical Research:

Hepatic receptors for glycoproteins with different terminal sugar residues may be of importance in the catabolism of glycoproteins and in the hepatic uptake of cells with modified glycoproteins on their surface membrane. The ability to manipulate the cellular distribution of an infused glycoprotein enzyme by modifying its carbohydrate moiety may be of clinical importance in enzyme replacement therapy. For instance, in Gaucher's disease, in which there is a deficiency of the enzyme glucocerebrosidase, glucocerebroside accumulates in Kupffer cells. Increased uptake of infused glucocerebrosidase by Kupffer cells may be achieved by exposing mannose as the terminal sugar of its carbohydrate moiety. Receptor mediated hepatic clearance of lysosomal enzymes, such as β-hexosaminidase A, may be of physiologic importance in preventing the accumulation in the circulation of potentially autodestructive enzymes. Studies of the kinetics of the interactions of HBP with its specific ligands provide important insights into the properties of receptors in general, e.g. the recycling of receptors. The finding that a glycoprotein recognition system on hepatic endothelial cells can be modulated by glucose and a glucose-albumin conjugate suggests a mechanism for abnormal glycoprotein metabolism in diabetes mellitus.

Proposed Course:

Future studies include 1) an assessment whether the receptor (hepatic binding protein) on hepatocytes which specifically recognizes galactose-terminated glycoproteins is glucose sensitive and 2) determinations whether the glucose sensitive glycoprotein recognition system on hepatic endothelial cells recognizes spontaneously glycosylated proteins.

Publications:

1. Jones, E.A., Vierling, J.M., Steer, C.J.: Surface receptors on hepatic sinusoidal cells. In Popper, H., Bianchi, L., Gudat, F., Reutter, W. (Eds.): Communication of Liver Cells. Lancaster, England, MTP Press Ltd., 1981, pp. 211-227.

2. Jones, E.A., Summerfield, J.A.: Kupffer cells. In Arias, I.M., Popper, H., Shafritz, D., Schacter, W., Jacoby, W. (Eds.): The Liver: Biology and Pathobiology. New York, Raven Press, 1981, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,509-03 DDB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of patients who are chronic carriers of hepatitis B surface antigen (HBsAg)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. H. Hoofnagle	Senior Investigator	DDB:NIADDK
Other:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
	G. Y. Minuk	Clinical Associate	DDB:NIADDK
	G. M. Dusheiko	Clinical Associate	DDB:NIADDK
	D. F. Schafer	Clinical Associate	DDB:NIAMDD
	R. H. Purcell	Chief, Hepatitis Section	LID:NIAID
	S. M. Feinstone	Senior Investigator	LID:NIAID
	J. Gerin	Head, Molec. Virol. & Immunol. Div.	Georgetown University
	R. Johnson	Medical Officer	LID:NIAID
	S. E. Straus	Senior Investigator	LCI:NIAID

COOPERATING UNITS (if any)

LID and LCI, NIAID
Georgetown University School of Medicine

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with histologically confirmed chronic HBsAg positive hepatitis are being studied. A total of 78 patients have been evaluated and 37 followed for at least one year. Twenty to thirty percent of patients undergo spontaneous resolution of their chronic hepatitis disease activity each year. This resolution is associated with seroconversion from HBeAg to anti-HBe. Therapeutic trials of the antiviral drug, adenine arabinoside monophosphate (Ara-AMP) are being undertaken. In an iniital study in which a ten-day course of the drug was given there was a uniform and prompt decrease in the levels of circulating hepatitis B virus DNA-polymerase and DNA. However, in only 17% of patients was the response long lasting. As the amount of Ara-AMP given in this first trial may have been insufficient for an optimum response to have occurred, a second randomized controlled study is being undertaken to evaluate a 28-day course of therapy. The role of immunologic mechanisms in the development of the chronic HBsAg carrier state is being investigated.

Project Description:

Objectives:

1) This study is designed to establish a group of patients with well characterized chronic HBsAg-positive hepatitis in preparation for treatment trials with antiviral agents.

2) Experiments are also designed to determine if subjects in whom HBsAg persists after hepatitis B infection have specific immunologic defects, such as an impaired ability to synthesize anti-HBs or impaired helper or suppressor T cell function.

Methods Employed:

1) Radioimmunoassays have been established for the detection and quantification of hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), antibody to HBsAg (anti-HBs), antibody to hepatitis B core antigen (anti-HBc) and antibody to HBeAg (anti-HBe).

2) IgG and IgM classes of antibodies are separated using sucrose density gradient ultracentrifugation and measured by radioimmunoassays.

3) Hepatitis B virus DNA polymerase is measured by an enzymatic assay.

4) Techniques of DNA hybridization are being applied to measure specific hepatitis B virus DNA.

5) Needle biopsies of the liver are processed for light, immunofluorescence and electron microscopy.

6) Peripheral blood mononuclear cells (PBMC) are isolated by Sephadex anti-$(Fab')_2$ affinity chromatography. PBMC are cultured for 10 days in the presence of pokeweed mitogen and IgG, IgM, anti-HBs and anti-HBc measured in culture supernatants using radioimmunoassays.

7) To study the regulation of immunoglobulin synthesis by B cells in vitro B and T cells are incubated for 10 days in the presence of pokeweed mitogen and IgG and IgM measured in culture supernatants using enzyme-linked immunosorbent assays (ELISA). The T:B cell ratio in cultures is varied to study helper (low ratios, e.g. $<$ 1:1) and suppressor (high ratios, e.g. 10:1) effects. T cells are irradiated to abolish selectively suppressor T cells.

Major Findings:

1) Seventy-eight HBsAg-positive persons have been enrolled in this project. Thirty seven have been followed for at least one year. Only one of the 78 has lost HBsAg-reactivity during follow-up. However, many patients have demonstrated a sponteous improvement in their underlying chronic hepatitis. In following patients prospectively, we have found that these spontaneous improvements are preceeded by a change in serological markers in the serum. In every case of

spontaneous improvement (as judged by sequential serum aminotransferase determinations), serum hepatitis B virus DNA and DNA polymerase activity disappeared and a seroconversion occurred from HBeAg to anti-HBe. Thus, the viral infection appeared to change from an active to a latent form. This occurred in 15-25% of HBsAg-positive and HBeAg-positive patients each year. Furthermore, in following patients with positive serum HBsAg and normal serum aminotransferases, we have found spontaneous exacerbations of disease activity to be often preceeded by a re-appearance of hepatitis B virus DNA and DNA polymerase activity in the serum.

2) Fifteen patients have been followed who were being treated with corticosteroids with or without azathioprine. Of interest, none of these 15 developed normal serum aminotransferases or underwent a seroconversion from HBeAg to anti-HBe while on corticosteroids. However, when corticosteroids were withdrawn from these 15, 7 (47%) seroconverted from HBeAg to anti-HBe and a dramatic fall in serum aminotransferase levels recurred within 1-4 months of discontinuation of medication. These observations suggest that corticosteroids actually prevent spontaneous improvement from occurring in this disease. An alternate explanation is that treatment with corticosteroids followed by their withdrawal may lead to a spontaneous remission in this disease.

3) These studies on the natural history of chronic type B hepatitis were initiated as a prelude to the start of trials of anti-viral agents for this condition. Two such trials have been undertaken using the antiviral agent, adenine arabinoside monophosphate (Ara-AMP). Patients selected to be treated with this agent have been carefully monitored and pre-screened to establish that they are not on the verge of a spontaneous remission. This is assured by demonstrating that patients have high and stable levels of hepatitis B virus - as assessed by serial serum determinations of HBsAg titer, HBeAg, DNA-polymerase and hepatitis B virus DNA. One such trial has been completed. Six patients with chronic type B hepatitis were treated with a ten-day course of Ara-AMP. All patients had an immediate response in that the levels of circulating hepatitis B virus DNA-polymerase and DNA decreased. However, only one patient (17%) had a long lasting response - the viral markers became negative and remained negative, seroconversion from HBeAg to anti-HBe occurred and the serum levels of aminotransferases fell to normal values. The remaining five patients had only a transient response, the serum DNA polymerase and DNA levels returning to pre-treatment levels as soon as the drug was stopped. As the amount of Ara-AMP given in this first trial might have been insufficient for an optimum response to have occurred, a second study was undertaken to evaluate a 28 day course of therapy. This is a randomized controlled trial. Fifteen patients have been admitted to the study and eigth have received Ara-AMP. So far, only one of the eight treated patients has had a long lasting response to therapy. In no patient in the control group has there been a loss of DNA polymerase or seroconversion from HBeAg to anti-HBe.

4) While effective antiviral treatment of chronic type B hepatitis remains an important goal, treatment of acute, uncomplicated type B hepatitis with antiviral agents is probably not feasible since maximal DNA polymerase activity, which is indicative of maximal viral replication, occurs before clinical symptoms become severe or serum aminotransferase levels reach peak values.

5) An epidemiological study of hepatitis B viral markers in an isolated Canadian Inuit community has indicated that hepatitis B virus infection was common in the community 20-30 years ago and that infection occurred predominantly during infancy, either as a result of maternal-infant transmission or post-natal exposure. During the past 20 years transmission of type B hepatitis to children in this community has become uncommon.

6) The mechanism by which some individuals who become infected with the hepatitis B virus fail to clear the virus and develop chronic type B hepatitis is being investigated. Failure to detect anti-HBs in chronic HBsAg carriers may be due to complexing of anti-HBs by excess circulating HBsAg or to a defect in the synthesis of anti-HBs. To examine the latter of these possibilities pokeweed mitogen-induced synthesis of anti-HBs, anti-HBc, IgG and IgM by peripheral blood mononuclear cells from HBsAg carriers and controls has been measured in vitro. The results indicate that lymphocytes from HBsAg carriers have an unimpaired ability to synthesize both IgG and IgM and are able to synthesize detectable amounts of anti-HBc. However, they do not synthesize measurable amounts of anti-HBs in vitro despite continuous antigenic exposure to HBsAg in vivo. The potential role of abnormal regulation of the immune response in the pathogenesis of chronic type B hepatitis is also being studied. T lymphocyte-mediated help and suppression of pokeweed mitogen-induced immunoglobulin (Ig) synthesis by cultured type B lymphocytes has been quantitated in vitro. At a T:B cell ratio of approximately 1:1 maximum Ig synthesis by cultures of cells from normal individuals occurs (helper effect) and at higher T:B cell ratios progressively less Ig is synthesized (suppressor effect). In contrast to cultures of cells from normal individuals, cultures of cells from many patients with chronic type B hepatitis continue to synthesize progressively larger amounts of Ig with increasing T:B cell ratios indicating a defect in suppressor cell function in these patients. Following irradiation of T cells to selectively destroy suppressor T cells, there appeared to be no difference in Ig synthesis by cultures of cells from patients with chronic type B hepatitis and normal subjects indicating that helper T cell function was normal in the patients.

Significance to Biomedical Research:

One goal of this project is to investigate the natural history of chronic HBsAg positive hepatitis and to execute appropriately designed trials of antiviral agents in this disease. As shown, there is a spontaneous remission rate for this disease of approximately 15-25% each year. Obviously, trials of antiviral agents will need to include sufficient numbers of treated and untreated persons to ensure that the effect of treatment is to induce a remission rate which is greater than the spontaneous remission rate for this disease. Studies such as this will also help to identify which patients are likely to benefit most from therapy. Patients currently considered most suitable for therapy with anti-viral agents are those who have stable levels of hepatitis B virus markers - HBeAg, HBsAg and DNA polymerase - and who have chronic active hepatitis as desmontrated by percutaneous liver biopsies.

Another goal of this project is to determine the mechanism by which some patients infected with the hepatitis B virus develop the chronic hepatitis B virus carrier state.

Proposed Course:

We plan to continue following a large number of HBsAg positive individuals in order to define the spontaneous rate of remission of this disease and to characterize the relationships between immunological features, hepatitis B virus serological markers and the clinical course of disease. Patients who demonstrate stable levels of hepatitis B virus markers will be eligible for anti-viral and immunological therapies. We have started a randomized, controlled trial of Ara-AMP in this disease in which patients will be treated for 28 days with both intravenous and intramuscular medication. Fifteen patients have been admitted to this trial. In the near future, we hope to start a randomized, double-blind trial of short-term, high-dose prednisolone therapy.

To investigate the nature of the suppressor cell defect in chronic type B hepatitis Ig synthesis by cultures containing complementary subsets of allogeneic immune cells from patients with chronic type B hepatitis and normal individuals is being determined. The patients being studied are from the group of patients with chronic type B hepatitis who will subsequently receive therapy with one or more antiviral agents such as Ara-AMP. Restudy of those patients who have responded successfully to therapy will permit identification of those defects of immune regulation which contribute to the development of chronic type B hepatitis and distinguish those which arise secondary to chronic hepatitis.

Publications:

1. Hoofnagle, J.H.: Type B hepatitis: Virology, serology and clinical course. Seminars in Liver Disease 1: 7-14, 1981.

2. Hoofnagle, J.H., Dusheiko, G.M., Seeff, L.B., Jones, E.A., Waggoner, J.G., Bales, Z.B.: Seroconversion from hepatitis B e antigen (HBeAg) to antibody (anti-HBe) in chronic type B hepatitis. Ann. Intern. Med. 94: 744-748, 1981.

3. Hoofnagle, J.H.: Chronic hepatitis. The role of corticosteroids. In Szmuness, W., Alter, H.J., and Maynard, J.E. (Eds.): Proceedings of the 1981 International Symposium on Viral Hepatitis. Franklin Institutes Press, Philadelphia, 1981, (in press).

4. Hoofnagle, J.H.: Serological markers of hepatitis B virus infection. Ann. Rev. Med. 32: 1-11, 1981.

5. Hoofnagle, J.H.: Hepatitis B surface antigen (HBsAg) and antibody (anti-HBs). In Bianchi, L., Gerok, W., Sickinger, K., and Stalder, G.A. (Eds.): Virus and the Liver. MTP Press, Lancaster, England, 1980, pp. 27-37.

6. Hoofnagle, J.H.: Therapy of acute viral hepatitis. In Conn, H.O. (Ed.): Proceedings of an International Symposium on (+)-Cyanidanol-3. Karger, Basel, 1981, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 53,510-02 DDB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of the Natural History of Chronic Non-A, Non-B Hepatitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. H. Hoofnagle	Senior Investigator	DDB:NIADDK
Other:	H. J. Alter	Chief, Immunology Section	BB:CC
	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
	G. M. Dusheiko	Visiting Associate	DDB:NIADDK
	D. F. Schafer	Clinical Associate	DDB:NIADDK
	R. H. Purcell	Chief, Hepatitis Section	LID:NIAID
	H. Denes	Visiting Scientist	LID:NIAID
	S. M. Feinstone	Senior Investigator	LID:NIAID
	K. Ishak	Chief, Hepatic Pathology Branch	A.F.I.P.
	G. Y. Minuk	Visiting Associate	DDB:NIADDK

COOPERATING UNITS (if any)

NIH Blood Bank
Hepatitis Section, LID:NIAID
Armed Forces Institute of Pathology

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with well-documented chronic non-A, non-B (non-lupoid) hepatitis are being evaluated to determine the natural history of this disease and to obtain tissues and reagents to search for non-A, non-B agents. Twenty-one patients have been admitted to this study. These patients have undergone thorough evaluation including a liver biopsy in twenty. They have had the disease for periods ranging from 1 to 8 years. Four patients have a mild degree of malaise, the remaining patients are asymptomatic. Liver biopsies have revealed chronic persistent hepatitis in five, chronic active hepatitis in eleven and cirrhosis in four. Examination of liver biopsies by electron and immunofluorescence microscopy has failed to show definitive evidence of viral antigens or viral particles. However, electron microscopy of 50% of biopsies has revealed intranuclear virus-particle-like structures which are similar to those observed in chimpanzees with non-A, non-B hepatitis. The significance and specificity of these structures is under investigation.

Project Description:

Objectives:

A third form of viral hepatitis - non-A, non-B - was first described in 1975. Since that time non-A, non-B hepatitis has been shown to be the most common type of post-transfusion hepatitis and to account for at least 20% of sporadic viral hepatitis. Non-A, non-B hepatitis from transfusion has a high propensity to progress to chronic hepatitis. The nature of the chronic hepatitis, its natural history, its therapy, its pathogenesis and its viral etiology remain poorly defined. The purpose of this study is to define the natural history to post-transfusion non-A, non-B hepatitis and to provide reagents (blood and liver biopsy tissue) for attempts to identify the agent(s) of non-A, non-B hepatitis.

Method Employed:

Patients followed in the post-transfusion hepatitis studies conducted by Dr. Harvey J. Alter (NIH Blood Bank) who develop non-A, non-B hepatitis and who have persistently elevated serum aminotransferase levels (ALT, AST) for more than 6 months are evaluated by the Liver Diseases Section. Patients undergo a comprehensive medical evaluation. Blood studies include HLA typing and collection of serum for agar gel diffusion and counterelectrophoresis tests for non-A, non-B antigens. If indicated, a percutaneous liver biopsy is obtained and the biopsy subjected to light, immunofluorescence and electron microscopy.

Major Findings:

Twenty-one patients have been evaluated and twenty have undergone percutaneous liver biopsy. Patients have had evidence of hepatitis for periods of time varying between 1 and 8 years. Four patients experience mild degrees of lethargy or malaise. The remaining patients have no symptoms attributable to liver disease. In contrast to chronic type B hepatitis serum aminotransferase levels fluctuate over a wide range, i.e. from normal values to as high as 700 I.U./1. So far, HLA typing has not revealed any correlations of note. In particular, the incidence of B8, DrW 3 types, which is increased in patients with autoimmune (lupoid) chronic hepatitis, is not increased in this population of patients with non-A, non-B hepatitis. Liver biopsies have revealed chronic active hepatitis in 11 patients, chronic persistent hepatitis in five and cirrhosis in four. Follow-up liver biopsies from eight patients, demonstrated progression of the liver disease in three and milder degrees of hepatocellular disease in the rest.

Immunofluorescence studies on the liver biopsies using sera from patients who have recovered from non-A, non-B hepatitis have not revealed any potential non-A, non-B antigens. Furthermore, electron microscopy of the biopsies has not demonstrated viral antigens or definite viral particles in the liver biopsies. However, 50% of biopsies have shown peculiar intranuclear clusters of pleomorphic particles. Similar structures have also been seen in liver biopsies from chimpanzees with non-A, non-B hepatitis. The significance and specificity of these nuclear changes are being investigated. In the course of assaying for antigens and antibodies specific for non-A, non-B hepatitis agar gel diffusion studies of sera from these patients have demonstrated a precipitin system similar to that described by other workers in this field. However, evaluation of this system has demonstrated that it is not due to an antigen-antibody reaction and that it is found in liver diseases other than non-A, non-B hepatitis.

Significance to Biomedical Research:

Non-A, non-B hepatitis is the most common form of post-transfusion hepatitis and occurs in 3-10% of transfused individuals. At present there are no markers for this disease and no associated non-A, non-B specific viral antigens or particles that have been definitively identified. Chronic liver disease due to non-A, non-B hepatitis is also common. There is no known therapy for this chronic liver disease. Corticosteroids do not seem to be of benefit. This study is aimed at identifying a viral antigen or marker that might be used to screen blood for the presence of this virus. This study is also designed to characterize the natural history of this disease, so that persons who need to be treated for their liver disease can be readily identified.

Proposed Course:

We plan to continue to follow the patients already admitted to this study and to admit further patients with chronic non-A, non-B hepatitis as they are identified in the post-transfusion hepatitis studies conducted at the NIH. The major areas of proposed study will be immunofluorescence and electron microscopy of liver biopsies and development of radioimmunoassays or enzyme-linked immunoassays for the detection of non-A, non-B antigens and antibodies.

Publications:

1. Hoofnagle, J.H.: Acute non-A, non-B hepatitis. Clinical course and diagnosis. In Gerety, R.J. (Ed.): Non-A, Non-B Hepatitis. Academic Press, New York, 1981 (in press).

2. Hoofnagle, J.H., Feinstone, S.M.: Serological tests for non-A, non-B hepatitis. Controversey. Liver 1: 1981, (in press).

3. Hoofnagle, J.H.: An immunoprecipitin system detected in sera from patients with non-A, non-B hepatitis. J. Med. Virol. 1981, (in press).

4. Dusheiko, G.M., Jones, E.A.: Managment of non-A, non-B hepatitis. In Gerety, R.J. (Ed.): Non-A, Non-B Hepatitis. Academic Press, New York, 1981, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,511-02 DDB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Controlled Trial of Chlorambucil Therapy in Primary Biliary Cirrhosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. H. Hoofnagle	Senior Investigator	DDB:NIADDK
	D. F. Schafer	Clinical Associate	DDB:NIADDK
	G. M. Dusheiko	Clinical Associate	DDB:NIADDK
	G. Y. Minuk	Clinical Associate	DDB:NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS: 2	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Symptomatic primary biliary cirrhosis (PBC) appears to be a uniformly fatal disease characterized by slowly progressive intrahepatic cholestasis due to a chronic non-suppurative destructive cholangitis involving the septal and larger interlobular bile ducts. The disease is associated with multiple defects of immunologic function, hepatic copper overload and a high incidence of various autoimmune diseases. Trials of corticosteroids and azathioprine have not shown that these agents effectively inhibit the progression of the disease or increase survival. Because certain chronic inflammatory diseases appear to respond favorably to alkylating agents but not to azathioprine, a controlled randomized trial of an alkylating agent for the treatment of PBC has been initiated. The alkylating agent chosen for the trial was chlorambucil. So far 16 patients have been admitted to the trial. Each patient undergoes a comprehensive medical evaluation prior to randomization and again after being in the trial for one and two years. Each evaluation includes a needle biopsy of the liver. In those receiving the drug the polymorphonuclear leucocyte count is maintained above 1000/mm^3. Five patients receiving the drug have had follow-up liver biopsies which show a marked decreased in inflammatory activity.

Project Description

Objectives:

The objective is to conduct a controlled trial of therapy with the alkylating agent chlorambucil in patients with primary biliary cirrhosis (PBC) to determine if therapy with this agent decreases the rate of progression of the disease process and increases survival.

Methods Employed:

1) Sixteen patients with clinical, serum biochemical, serologic and hepatic histologic features consistent with the diagnosis of PBC have been admitted to the trial. Patients must have symptoms attributable to PBC. Reasons for excluding patients include: over 65 years old, a serum bilirubin greater than 10 mg/dl and clinical features of decompensated chronic hepatocellular disease.

2) Patients undergo an initial comprehensive medical evaluation, which includes determinations of indices of cholestasis and hepatocellular function, serum levels of lipoproteins, autoantibodies, immune complexes and bile acids, and a needle biopsy of the liver. They are then randomized into a group which receives chlorambucil and a group which receives no chlorambucil, by opening sealed envelopes. Patients in both groups receive all of the conventional supportive treatments for the various manifestations of PBC as indicated. The dose of chlorambucil is adjusted so that the polymorphonuclear leucocyte count is maintained above $1000/mm^3$.

3) All patients are subjected to further comprehensive medical evaluations, which include repeat needle biopsies of the liver, after being in the trial for one year and two years.

Major Findings:

In patients admitted to the trial who are receiving chlorambucil, lymphocytopenia has been induced. Five patients receiving the drug have had follow-up liver biopsies which show a substantial decrease of inflammatory activity. It is too early in the course of the trial to determine whether chlorambucil therapy has any definite beneficial effects on the course of patients with PBC.

Significance to Biomedical Research:

Primary biliary cirrhosis (PBC) when symptomatic appears to be a uniformly fatal disease characterized by slowly progressive intrahepatic cholestasis due to a chronic non-suppurative destructive cholangitis involving the septal and larger interlobular bile ducts. The disease is associated with multiple defects of immunologic function suggestive of autoimmunity as well as hepatic copper overload. Trials of corticosteroids and azathioprine have not shown that these agents effectively inhibit the progression of the disease or increase survival.

Trials of penicllamine suggest that there may be some improvement in the mortality rate if the drug is given to patients with advanced stages of the disease. However, penicillamine treatment in this disease is associated with a high incidence of serious side effects and many patients cannot tolerate this drug. Because certain chronic inflammatory autoimmune diseases appear to respond favorably to alkylating agents but not to azathioprine, a controlled randomized trial of an alkylating agent for the treatment of PBC has been initiated. The alkylating agent chosen for the trial was chlorambucil rather than cyclophosphamide because of the less severe toxicity associated with the use of the former drug.

Proposed Course:

It is anticipated that the trial will take a period of approximately 3-5 years to complete.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53,512-02 DDB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of the Uptake of Lipoproteins by Hepatic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK
Other:	J. A. Summerfield	Visiting Scientist	DDB:NIADDK
	J. Vergalla	Chemist	DDB:NIADDK
	E. Schaefer	Senior Investigator	MD:IR:NHLBI
	B. Brewer	Chief, Mol. Dis. Br.	MD:IR:NHLBI

COOPERATING UNITS (if any)

Molecular Diseases Branch, NHLBI

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS X☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are being designed to determine which of the major classes of hepatic cells are responsible for the uptake of different lipoproteins by the liver and to define which apoproteins mediate the uptake of lipoprotins by the different cell types. Rat endothelial, Kupffer and parenchymal cells have been isolated by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. Apoproteins isolated from normal subjects and patients with Tangier disease were radioiodinated and reassociated with lipoprotein particles from normal subjects and patients with Tangier disease. Measurements of the uptake of these labeled particles by hepatic cells in vitro indictate that the increased reticuloendothelial (RE) cell uptake of cholesterol in Tangier disease is probably mediated by enhanced uptake of Tangier apoprotein A-I-containing cholesterol-rich lipoproteins by RE cells and that the rapid clearance of lipoproteins from the plasma in Tangier disease can be accounted for by the uptake of Tangier aproprotein A-I-containing lipoproteins by hepatocytes.

Project Description:

Objectives:

The main objectives of this project are to determine which of the major classes of hepatic cells (parenchymal, endothelial, Kupffer) are responsible for the uptake of different lipoproteins by the liver, to define which apoproteins mediate the uptake of lipoproteins by the different cell types, and to assess whether aproproteins in certain disease states mediate abnormal cell uptake and/or catabolism of lipoproteins.

Methods Employed:

1. Suspensions of hepatic cells are generated by perfusing rat livers in situ with a solution of collagenase. The basic technique has been modified by including in the perfusate soybean trypsin inhibitor to reduce tryptic damage to cell surface receptors and deoxyribonuclease to inhibit DNA-mediated aggregation of cells.

2. Endothelial and Kupffer cells are separated gently from erythrocytes and parenchymal cells by Percoll gradient centrifugation.

3. Endothelial cells are separated from Kupffer cells by centrifugal elutriation.

4. The viability of cell preparations is assessed by measuring cell membrane potentials.

5. Lipoproteins are isolated from rat and human serum by preparative ultracentrifugation.

6. Apoproteins are isolated using column chromatography

7. Isolated apoproteins are labeled with radioiodine using the iodine monochloride or chloramine T technique and are allowed to reassociate with lipoprotein particles. Uptake of these labeled particles by hepatic cells in vitro at 37°C is quantitated.

Major Findings:

Familial high density lipoprotein (HDL) deficiency (Tangier disease) is characterized by low levels of plasma HDL and apolipoprotein (apo) A-I and A-II, rapid and abnormal catabolism of apoA-I and A-II and cholesterol-laden reticuloendothelial (RE) cells. Furthermore, in Tangier disease apoA-I and A-II are predominantly associated with lipoproteins of density < 1.063 g/ml and not with HDL. To investigate the molecular basis of these abnormalities the uptake by isolated rat hepatic cells of apoA-I and A-II isolated from normal subjects and Tangier homozygotes, radioiodinated and reassociated with lipoproteins of density < 1.063 g/ml has been studied. Uptake of labeled lipoproteins by cells was inhibited by dilution with cold ligand indicating that uptake was specific. The results indicated that there was preferential uptake of lipoproteins containing apoA-I from patients with Tangier disease by both Kupffer cels and hepatocytes.

In contrast, there was no preferential uptake of lipoproteins containing apoA-II from patients with Tangier disease by either Kupffer cells or hepatocytes. Uptake of labeled ligands by endothelial cells was negligible. These findings indicate: 1) the increased RE uptake of cholsterol-laden lipoproteins in Tangier disease is probably mediated by enhanced uptake of ApoA-I (Tangier)-containing liporoteins, 2) since Kupffer cells and hepatocytes account for about 8% and 66% of total liver cells, respectively, the abnormally rapid disappearance of certain lipoproteins from plasma in Tangier disease can be accounted for by parenchymal cell uptake of apoA-I (Tangier)-containing lipoproteins.

Significance to Biomedical Research:

Abnormal levels of low and high density lipoproteins in the blood have been implicated in the pathogenesis of atherosclerosis. Factors responsible for regulating levels of lipoproteins in the blood include synthesis by the intestine and liver and uptake and catabolism by the liver and peripheral tissues. This protocol is designed to examine the mechanisms involved in the catabolism of lipoproteins by the liver under normal physiologic conditions and in pathologic states. Initial studies have clarified the molecular basis of Tangier disease by demonstrating that a structurally abnormal apoA-I is responsible for mediating abnormal lipoprotein metabolism in this disease.

Proposed Course:

Future studies, using the same experimental approach as was used to investigate lipoprotein metabolism in Tangier disease, will evaluate the role which different apo E's and abnoraml apolipoproteins play in the pathogenesis of hyperlipidemias.

Publications:

1. Jones, E.A., Summerfield, J.A.: Kupffer cells. In Arias, I.M., Popper, H., Schafritz, D., Schacter, D., Jacoby, W. (Eds.): The Liver: Biology and Pathobiology. New York, Raven Press, 1981, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 53,513-01 DDB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies of the Role of Chemotaxis in the Pathogenesis of Hepatic Fibrosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	W. G. Cooksley	Visiting Scientist	DDB:NIADDK
Other:	G. Grotendorst	Post-doctoral Fellow	KDBA:NIDR
	C. McIvor	Guest Worker	DDB:NIADDK
	J. Summerfield	Guest Worker	DDB:NIADDK
	J. Vergalla	Chemist	DDB:NIADDK
	E. A. Jones	Chief, Liver Diseases Section	DDB:NIADDK

COOPERATING UNITS (if any)

Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The fibrosis in the liver which can occur pari passu with chronic hepatocellular injury may arise as a result of increased collagen synthesis by fibroblasts and smooth muscle cells which have migrated to the liver in response to the release of chemotactic factors. The nature of these putative chemotactic factors, their cell of origin and the sequence of events which leads to their release and biological activity are unknown. Using an in vitro assay to measure the response of collagen synthesizing cells to chemotactic factors evidence for the production of these factors is being sought in an animal model of hepatic fibrosis and in patients with chronic hepatocellular disease.

Project Description:

Objectives:

The objective of this protocol is to explore factors involved in hepatic fibrogenesis, a pathologic process which precedes the development of cirrhosis. It is believed that chemotactic substances are released pari passu with chronic hepatocellular injury and that these substances cause smooth muscle cells and fibroblasts to migrate to the liver, proliferate and synthesize collagen with the formation of fibrous tissue. This project is designed to determine whether such chemotactic substances are released in an animal model of hepatic fibrosis and in patients with chronic hepatocellular diseases, and whether they are generated by injured hepatocytes or by immunocompetent cells which are mediating the hepatocellular injury. An understanding of the pathogenesis of hepatic fibrosis may lead to means of preventing or inhibiting the development of cirrhosis.

Methods Employed:

1) Hepatic fibrosis is induced in rats by chronic administration of carbon tetrachloride and phenobarbital.

2) Hepatocytes, Kupffer cells and endothelial cells are isolated from normal rats and rats with hepatic fibrosis by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation.

3) Kupffer cells, endothelial cells and hepatocytes are cultured overnight to produce conditioned media.

4) Homogenates of normal rat liver and of fibrotic liver are prepared.

5) Slices of normal liver and fibrotic liver are incubated.

6) Peripheral blood mononuclear cells from patients with liver disease are cultured in the presence of polyclonal mitogens, pokeweed mitogen and phytohemagglutinin and specific disease-related antigens such as HBsAg.

7) Conditioned media, liver homogenates, liver slice supernatants and supernatants of cultured lymphocytes are assayed for factors that are chemotactic to ovine vascular smooth muscle cells and human fibroblasts by measuring the degree to which their presence in a Boyden chamber induces these cells in culture to migrate across a pore-containing membrane.

Significance to Biomedical Research:

Hepatic fibrosis is a major component of the disturbed hepatic architecture in most chronic liver diseases. In cirrhosis it is associated with the development of portal hypertension and episodes of bleeding into the gastrointestinal tract from esophageal varices. This project is designed to investigate the pathogenesis of the abnormal laying down of collagen and fibrosis in the liver in chronic liver disease. The results obtained may suggest means of decreasing the rate of progression of hepatic fibrosis or preventing it altogether.

Proposed Course:

Studies are being carried out as described above.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54016-06 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Lipoprotein Composition in Indians and Caucasians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.V. Howard	Assistant Chief	PCRS, NIAMDD
OTHER:	S.L. Aronoff	Chief	PCRS, NIAMDD
	J. Reitman	Staff Physician	PCRS, NIAMDD
	P.H. Bennett	Chief	EFSB, NIAMDD
	W.C. Knowler	Chief	SFSS, NIAMDD
	D.J. Pettitt	Assistant Chief	SFSS, NIAMDD
	J.L. Lisse	Staff Physician	SFSS, NIAMDD

COOPERATING UNITS (if any) E. Nikkila, M. Taskinen, Univ. of Helsinki, Finland; R.H. Unger, M.D., U. of Texas, Southwestern Medical School, Dallas TX; J. Fuller and M. Mattock, Guys Hospital, London England D. Steinberg, U. of California San Diego, CA

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	0.4	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Both low density lipoproteins and very low density lipoproteins have been implicated as positive risk factors in the genesis of atherosclerosis, whereas high density lipoprotein concentration is an independent negative risk factor. Both diabetes and obesity are associated with changes in plasma lipoproteins and these changes may be related to the increased prevalence of cardiovascular disease. We are studying lipoprotein composition in obese and diabetic Pima Indians. Cholesterol and triglycerides are being measured in high density lipoprotein as well as other lipoprotein subclasses isolated by ultracentrifugation. These parameters are being compared in groups of normal and diabetic Indians with and without coronary artery disease, and the influence of therapy is being assessed. Apoprotein composition of specific lipoproteins will be measured. In addition, free fatty acid levels are being studied because of their precursor relationship to lipoproteins and their reflection of insulin action. From this study we will attempt to determine if diabetes and obesity are associated with changes in serum lipoproteins and to delineate the relation between lipoprotein levels and the pathogenesis of ischemic heart disease.

PHS-6040
(Rev. 10-76)

	(A) No. on Reservation as of 1/80	(B) No. seen in clinic 11/78-3/81	B as percent of A	(C) No. with lipids 11/78-3/81	C as Percent of B
Males					
15-24	470	228	49%	152	-
25-34	304	124	41%	103	83%
35-44	199	103	52%	86	83%
45-54	133	89	67%	85	96%
55-64	85	45	53%	27	60%
$\geq$65	116	59	51%	49	83%
Females					
15-24	503	384	76%	274	-
25-34	304	231	76%	193	84%
35-44	199	164	82%	131	80%
45-54	182	168	92%	143	85%
55-64	117	99	85%	83	84%
$\geq$65	110	73	66%	58	79%

2. Relationship Between Diabetics and the Risk of Atherosclerotic Heart Disease (ASCVD) in the Pima Indians.

a. Prevalence of EKG abnormalities ASCVD. The relationship between diabetics and ASCVD in this population has been examined using data obtained from the standard 12-lead EKG at the biennial examination. EKG abnormalities were defined according to the Tecumseh criteria. In diabetic Pimas, the prevalence of EKG abnormalities was 2.1 times that of the nondiabetic Pimas. This observation was supported by determination of the frequency of cardiovascular disease (defined as sudden death with greater than 75% stenosis of at least one coronary artery, myocardial infarction, or stroke) among Pimas at autopsy. This autopsy analysis was restricted to males who were at least 55 years old at death, because there were few cardiovascular deaths in females and younger males. The post-load glucose concentration was determined at the last examination prior to death. Although the study was too small to be conclusive, cardiovascular disease was the cause of death 1.8 times as frequently in the diabetics; a prevalence ratio similar to that for EKG abnormalities in living subjects.

The relationship between hyperglycemia and the development of ASCVD has been examined in a prospective study of 590 subjects at least 25 years old with no EKG abnormalities at the initial examination as defined by the Tecumseh

Project Description:

Objectives:

1. Determination of Lipoprotein composition in normal and diabetic Indians: Previous studies have yielded data on total serum cholesterol and triglyceride levels in the the Pima Indian population. In this study lipoprotein classes will be isolated by ultracentrifugation and cholesterol, triglyceride,and apoproteins quantified in the isolated lipoproteins. These parameters will be compared in normal and diabetic individuals, and in diabetics before and after therapy to determine if diabetes is associated with changes in lipoprotein composition and how these changes are related to therapy and complications.

2. Studies of the relationship between high density lipoprotein composition and ischemic heart disease: Recent studies have suggested that there may be a relationship beteen HDL levels and atherosclerotic heart disease. Our objective is to quantify HDL levels and study HDL composition in a series of normal and diabetic Indians with and without coronary artery disease. Study of HDL in this homogeneous and well characterized population should clarify the role of this lipoprotein in the etiology of atheroscleros especially in obese and diabetic individuals.

Major Findings:

1. Determination of lipoprotein composition in normal and diabetic Indians:

a. Cross sectional study of lipoprotein composition and HDL subfractions in the Pima Indians: Blood sampling to measure lipoprotein triglycerides and cholesterol has continued on all individuals over age 15 seen for biennial exams at SFSS. In addition, HDL subfractions are being isolated from all individuals between 35-55 years. Cholesterol and triglycerides have been measured in all lipoprotein fractions and the fractions have been stored frozen for future apoprotein studies. We have participated in a standardization program with the CDC, Atlanta, Georgia, in order to insure that our plasma lipid measurements will be comparable to those of the Lipid Research Clinics. In collaboration with Drs. Fuller and Mattock in London and Drs. Nikkila and Taskinen in Helsinki comparable groups of Caucasian diabetics have been recruited and plasma lipoproteins are being analyzed. To insure comparability of methods, plasma samples and cholestero and triglyceride controls have been exchanged at approximately six month intervals. To date approximately 1400 Pima samples have been quantified over the following sex and age ranges:

criteria. At follow-up examination after 6 years (range 5-7 years) all subjects were reassessed for EKG abnormalities. EKG abnormalities occurred 1.7 times as frequently in diabetics as in nondiabetics. This was similar to the prevalence ratios for EKG abnormalities and cardiovascular disease at autopsy, but was in striking contrast to the greatly increased incidence of retinopathy in diabetics. Thus, in this population with relatively low prevalence of ASCVD diabetes is associated with an increase in cardiovascular disease. The relationship to other risk factors in the population must be examined further.

b. Relationship Between Plasma Lipids and ASCVD in Pima Diabetics. Seventeen autopsy cases were identified with ASCVD as described above. Post-mortem cases without evidence of ASCVD were matched for age (decade), sex, weight (within 5 Kg), and diabetic status and treatment. Frozen serum was available which had been drawn from these individuals 1 to 11 years prior to death. HDL were isolated by the heparin-manganese precipitation method, and HDL and total cholesterol were determined on the Autoanalyzer. Assays were standardized using reference material supplied by the CDC Lipid Standardization Laboratory (Atlanta, Georgia). Previous investigation of six healthy volunteers indicated that storage of frozen samples for up to one year did not affect plasma HDL concentrations (51.7±11.2 mg/dl, mean ± SD vs 52.7±11.6 after freezing, r=.99) and that up to 10 freeze-thaws also had no significant influence on HDL concentrations (51.7±11.2, mean ± SD vs 53.7±10.9 after freezing, r=.99).

In nondiabetics mean HDL cholesterol concentration tended to be lower in those with ASCVD and the ratio of total/HDL cholesterol was somewhat higher. In autopsied diabetics the mean total cholesterol concentration was higher in those with ASCVD, but HDL levels in diabetics with evidence of ASCVD were similar to the HDL levels in those who died without ASCVD.

The relationship between plasma lipid concentrations and prevalence of EKG abnormalities was next examined using fresh plasma samples obtained from fasting patients seen at biennial examinations during a one year period. Lipoproteins were isolated by ultracentrifugation and total and lipoprotein cholesterol and triglylcerides were measured on the Autoanalyzer. EKG abnormalities were defined using the Tecumseh criteria. Subjects with abnormalities were matched with paired controls selected from those with no evidence of EKG abnormalities and matched for age (decade), sex, weight, (within 5 Kg), diabetic status and diabetic treatment. In nondiabetics means for total cholesterol and total triglycerides were higher in those with abnormal EKGs, but the differences were not significant. HDL cholesterol levels and the ratio of total/HDL cholesterol were similar in nondiabetics with and without abnormal EKGs. In the diabetics, EKG abnormalities were associated with lower HDL cholesterol ($p<0.05$) and higher ratios of total/HDL cholesterol ($p<.01$). Diabetic subjects with abnormalities had higher triglycerides but the difference was not significant. These results indicate that the hyperlipemia associated with diabetes may be influential in the development of its macrovascular complications.

c. Comparison of Pima and Caucasian Diabetics: Individuals in the: Pima population with evidence of EKG abnormalities have been identified and plasma samples are being obtained at the Sacaton clnic from these individuals at the time of their biennial examination. Lipoprotein cholesterol and triglycerides are being measured and HDL subfractions isolated from these and from controls matched for age, sex, weight and diabetic status. Comparable groups of Caucasians with EKG abnormalities have been identified by Drs. Fuller and Mattock in London and Drs. Nikkila and Taskinen in Helsinki for similar lipoprotein quantification. To date 50 Pimas with EKG changes have been identified and analyzed.

Significance to Biomedical Research and the Program of the Institute: This study will determine whether changes in lipoprotein composition, and especially HDL cholesterol levels, are associated with diabetes and the development of ischemic heart disease. The data obtained on HDL subclasses will help elucidate the relationship of this lipoprotein to the development of diabetes and atherosclerosis. Since this population serves as a carefully defined model for adult onset diabetes, the data should contribute to an understanding of the effects of diabetes on lipoprotein metabolism and the relationship of lipoproteins to the development of both atherosclerosis and microvascular complications.

Proposed Course:

1. Determination of lipoprotein composition in normal and diabetic Indians.

a. Comparison of nondiabetics and diabetics. Assays of lipoprotein cholesterol and triglycerides and apoprotein levels in all individuals over age 15 seen for biennial exams will be continued in order to obtain enough samples for analyses by age groups within the normal and diabetic population. Data analysis will begin and results of Pima lipoprotein patterns will be compared to Caucasian diabetics selected and assayed by Drs. Fuller and Nikkla. Methods will be developed for assay of apoproteins B, C, E, and A so that data on lipoprotein lipids may be compared to that of apoproteins.

b. Studies on HDL. Measurements will continue of HDL composition in 35-54 year old diabetic and nondiabetic Pima Indians. In addition, a radioimmunoassay will be developed to measure apo A-1 and apo-2 levels in the HDL fractions and to compare these in HDL from nondiabetics and diabetics. Analysis of the data on individuals with EKG changes will begin and results will be compared to the Caucasian data.

c. Design of prospective study on plasma lipoproteins and the development of ASCVD in diabetics. In conjunction with the Epidemiology and Field Studies Section a prospective study will be designed and instituted to determine the relation between lipoprotein composition and the development of cardiovascular disease in diabetics. Several criteria in addition to EKG measurements will be instituted for the evaluation of cardiac and peripheral vascular disease. The basis for patient selection will be the large sample on whom baseline lipoprotein measurements have been accomplished.

Publications:

1. Howard, B.V., Lisse, J.R., Knowler, W.C., Davis, M.P., Pettitt, D.J., Bennett, P.H.: Diabetes and Atherosclerosis in the Pima Indians. In: Proceedings of a Workshop on Diabetes and Atherosclerosis. (Eds.) M. Ellenberg and V. Brown, in press, 1981.

2. Wasada, T., Howard, B., Dobbs, R.E., and Unger, R.H.: Evidence for a role of FFA in the regulation of somatostatin secretion in normal and alloxan diabetic dogs. J. Clin. Invest. 86:511-516, 1980.

3. Wasada, T., Howard, B., McCorkle, K., Harris, V., and Unger, R.: High plasma free fatty acid levels contribute to the hypersomatostatinemia of insulin deficiency. Diabetes 30:358-361, 1981.

4. Knowler, W.C., Savage, P.J., Nagulesparan, M., Howard, B.V., Pettitt, D.J., Aronoff, S.L., and Bennett, P.H.: Obesity, insulin resistance and diabetes mellitus in the Pima Indians. In: Proceedings of the Second International Conference on the Genetics of Diabetes Mellitus, in press, 1981.

Contract No. N01-AM-6-2219

Starting Date: September 1, 1980
Expiration Date: October 31, 1981

Contractor: University of Texas Health Science Center
Southwestern Medical School

Money Allocated: 2,794,619

Man Years Purchased: 32.2

This contract allowed extension of the studies carried out under the NIH Project Z01 AM 54016-06 DD.

Relation between fatty acid levels and somatostatin secretion. To investigate the effect of acute elevation of plasma free fatty acids on the secretion of splanchnic somatostatin-like immunoreactivity (in collaboration with Dr. Unger) the peripheral venous pancreatic and gastric venous effluent levels of SLI were measured in normal and chronic alloxan diabetic dogs before and after the infusion of a fat emulsion supplemented with heparin. In normal conscious dogs heparin, injected during the infusion of a fat emulsion, elevated FFA levels from a mean (± SE) baseline level of 0.7±0.1 mEq to a peak value of 1.5±0.1 mEq/l (p<.001) and plasma SLI rose from a mean (±SE) baseline value of 1.5±7 pg/ml to a peak of 253±44 pg.ml (p<0.05). Neither the infusion of glycerol, of fat emulsion without heparin, of heparin alone nor of saline itself had an effect on either the plasma level of FFA of SLI. In another group of anesthetized dogs with surgically implanted catheters the adminis tration of fat emulsion plus heparin was accompanied by more than a two-fold rise in the concentration of SLI in the venous effluent of the pancreas and of the gastric fundus and antrum in association with an elevation of FFA levels. In a group of conscious diabetic dogs fat emulsion plus heparin raised FFA from a mean baseline level of 1.2± 0.2 mEq/l to 1.6±0.3 mEq/l (p<.05) and SLI rose from a mean baseline level of 185±9 pg/ml to a peak value of 310±44 pg/ml (p<0.01). Although SLI levels were significantly greater than in normal dogs at several time points after the rise in FFA, the magnitude of the increment in diabetic dogs did not differ from normal. These result demonstrate that a rise in FFA levels is a potent stimulus for SLI secretion from the pancreas and stomach and raise the possibility that FFA is an important physiological regulator of SLI selection.

High plasma levels of free fatty acids (FFA) stimulate the secretion of splanchic somatostatin, and both are elevated in insulin deficiency. To determine if the hypersomatostatinemia of insulin deficienty is secondary to high FFA levels, plasma somatostatin-like immunoreactivity (SLI) was measured in a group of insulin-deprived alloxan diabetic dogs during nicotinic acid-induced lowering of their elevated plasma FFA to normal and in a group of nondiabetic dogs during nicotinic acid-induced lowering of the FAA to subnormal values. In insulin-deprived diabetic dogs, nicotinic acid reduced plasma FFA from 1.07±0.2 (M ± SE) mmol/l to 0.6±0.1 mmol/l (p<0.02), approximately the basal FFA level in normal dogs. This was accompanied by a significant decline in plasma SLI levels from a mean baseline of 247±15 pg/ml to a mean nadir of 199±10 pg/ml (p<0.005). The latter was, nevertheless, significantly above the basal SLI level of the nondiabetic dogs. In contrast, in normal dogs nicotinic acid-induced reduction in FFA from 0.54±0.02 mmol/l to 0.24±0.03 mmol/l (p<0.001) was associated with only a small and inconsistent decrease in SLI. These findings suggest that the hyper-matostatinemia of insulin deficient alloxan diabetic dogs is, in part, secondary to high plasma FFA levels.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM54017-05 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Weight-reduction on glucose tolerance and gluco-regulatory hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	J.S. Reitman	Staff Physician	PCRS, NIAMDD
OTHER:	S.L. Aronoff	Chief	PCRS, NIAMDD
	M. Nagulesparan	Guest Worker	PCRS, NIAMDD

COOPERATING UNITS (if any)
R.H. Unger, University of Texas Health Science Center, Dallas
A.H. Rubenstein, University of Chicago, School of Medicine, Chicago

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 4.3	PROFESSIONAL: 1.3	OTHER: 3.0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have shown that weight reduction improves insulin sensitivity and insulin secretory response to oral glucose in obese normal and diabetic subjects. Further studies designed to help elucidate the relationship of Type II diabetes to obesity have employed insulin therapy and exercise training. Short term (10 days) insulin therapy was shown to have no effect on sensitivity to insulin as measured by the glucose clamp technique, however, there was an improvement in the blunted insulin response to oral glucose. Six to twelve weeks of aerobic bicycle exercise resulted in improvement in maximal oxygen uptake capacity in obese normal, glucose intolerant and diabetic subjects. Exercise training resulted in improvement in insulin sensitivity in normal, glucose intolerant and mildly diabetic subjects, but no improvement was seen in moderately diabetic subjects. On the other hand, oral glucose tolerance improved in all diabetic subjects and could be attributed in part to an improvement in the early insulin response with exercise training in the diabetics.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Many components of the natural history of diabetes remain to be described. A major difficulty is the absence of sequential data on individuals in transition from the normal to the diabetic state. The study of obese subjects with borderline glucose tolerance compared to those with recent-onset and long-term diabetes is one approach to learning about the interrelationships of glucose tolerance and glucose regulatory hormone levels. Study of these different stages of diabetes employing various interventions such as weight loss, exercise or strict control of blood glucose with exogenous insulin therapy may reveal information concerning the reversibility of defects in insulin secretion and its regulation, and sensitivity to insulin at various stages in the development of diabetes.

Our studies of weight reduction have already revealed that oral glucose tolerance improves with improved insulin secretion in recent onset diabetics, but i.v. glucose tolerance remains impaired with lack of an acute insulin response. Subjects with impaired glucose tolerance demonstrate exaggerated insulin secretion in response to oral glucose before weight reduction and this decreases following weight loss in spite of improvement in glucose tolerance. With weight reduction in long-term (>5 years duration) diabetics, oral glucose tolerance and as well as the insulin secretory response to oral glucose response improve; however, the improvement in glucose tolerance is much poorer than in recent-onset diabetics, implying a greater degree of insulin resistance.

To extend these observations we have embarked upon a program to examine (1) the effects of "tight" control of blood sugar with insulin therapy in diabetes and (2) the effects of physical exercise on glucose tolerance and insulin secretion in diabetics.

Methods Employed: To study the effect of exogenous insulin treatment on glucose homeostasis in diabetes, seven obese Type II diabetics were placed on a 45% CHO, 35% fat, 20% protein weight maintenance diet and treated with four daily injections of regular insulin in order to maintain the plasma glucose near 100 mg/dl. Subjects were studied before and after insulin treatment with a 75 gm oral glucose tolerance test and a test of insulin sensitivity employing a constant insulin infusion with variable glucose infusion to maintain blood sugar at 100 mg/dl (glucose clamp).

Six obese Type II diabetics and seven obese nondiabetic controls took part in an exercise study, in which the subjects engaged in daily, intermittent, aerobic bicycle exercise. The diabetics each underwent a 75 gm oral glucose tolerance test, euglycemic glucose clamp (GC), 24 hour urinary glucose and C-peptide determinations, and body density measurement (underwater weighing). Five of the six diabetics also had maximal oxygen uptake determination before and throughout the training period to monitor physiologic improvement with training.

Major Findings:

1. Effect of Control of Glucose with Insulin Therapy. Control of plasma glucose levels in seven obese Type II diabetics by regular insulin injection (60-205 U/day in four divided doses) resulted in significant improvement in plasma insulin and C-peptide responses to oral glucose. After 10 days of therapy insulin induced glucose disposal rates (M values) estimated by the glucose clamp technique were unchanged. In two diabetics whose therapy was extended to 30 days, M values increased. These results indicate that short-term control of plasma glucose improve insulin secretion but not insulin sensitivity and that prolonged control may improve both. The impaired insulin secretion and insulin sensitivity in Type II diabetics appears to be in part secondary to metabolic abnormalities associated with hyperglycemia.

2. Effects of Physical Exercise. Six obese nondiabetic controls (two had mildly elevated 2 hour glucose values (185-190) were studied. Of the four who have completed the OGTT before and after exercise oral glucose tolerance improved slightly in all four and insulin secretory response to oral glucose was essentially unchanged in all. Three have completed the GC before and after exercise. Of these two showed marked improvement in insulin sensitivity and one was unchanged.

Of the diabetic subjects, one was borderline (FBS = 110, 2 hour glucose = 217), two were mild (FBS 140),two were moderate (FBS = 170-200) and one was severe (FBS 250). The severe diabetic has not yet completed the protocol. All diabetics showed substantial improvement in exercise capacity and VO_2 max. All of the diabetics achieved better control of blood sugar while maintaining weight during the exercise program,and glucose tolerance improved. Thirty minute insulin secretory response was improved in all, but the integral of the insulin responses to oral glucose was unchanged. The one borderline and one of the mild diabetics demonstrated improvement in insulin sensitivity by GC with training. None of the other diabetics showed improvements in insulin sensitivity, although glucose tolerance was improved in all.

Body density determinations appear to indicate that most subjects have a slight exchange of fat for lean body mass (~1 Kg) after exercise training, although such a change is well within the variability of the method.

The results to date indicate that the response of in vivo sensitivity to insulin (GC) in obese individuals to exercise training appears to depend in large part upon the degree of abnormality in glucose tolerance, with well-established diabetics showing the least response of insulin sensitivity to training. On the other hand improvement in control of plasma glucose occurred in all the diabetics studied. Furthermore, the more severe diabetics appeared to have a greater return of insulin secretory response to oral glucose. This is similar to the results of 10 day treatment with exogenous insulin in the first study, in which insulin sensitivity was unchanged, but secretory response to oral glucose improved. Thus, it appears that exercise can improve disposal of blood glucose sufficiently (in spite of caloric intake) to improve overall plasma glucose control. Improved control by whatever means (insulin treatment, weight loss or exercise) appears to improve the insulin response to oral caloric stimuli.

Significance to Biomedical Research and the Program of the Institute: Weight reduction, exogenous insulin treatment and exercise training all appear to improve certain metabolic papameters which define the diabetic state. Besides simple improvement in plasma glucose control, these interventions also result in improvements in receptor binding and insulin sensitivity (with weight reduction) and insulin response to oral glucose (weight reduction, exercise, and insulin treatment).

Obese normal and glucose intolerant subjects are known to benefit from weight reduction (improvement in receptor binding and insulin sensitivity and a more normal insulin response to oral glucose). It now appears that exercise training also can improve glucose tolerance and insulin action in these groups and could be used as an adjunct to weight control for the treatment of these conditions.

These findings are expected to increase understanding of the relationship between obesity and diabetes and improve methods of treating these conditions.

Proposed Course: We plan to complete the studies of exercise in those patients still engaged in the protocol in order to extend our findings. 24 hour C-peptide excretion will be measured to give additional insight into the functioning of the β-cell before and after exercise or weight loss. Studies of lipid metabolism in obese normal subjects before and after exercise are also in progress.

Publications:

Nagulesparan, M., Savage, P.J., Bennion, L.J., Unger, R.H., and Bennett, P.H.: Diminished effect of caloric restriction on control of hyperglycemia with increasing known duration of Type II diabetes mellitus. J. Clin. Endocrinol. Metab., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 54018-05 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Characterization of Metabolic and Morphologic Abnormalities in Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.L. Aronoff	Chief	PCRS, NIAMDD
OTHER:	M. Nagulesparan	Guest Worker	PCRS, NIAMDD
	J.S. Reitman	Staff Physician	PCRS, NIAMDD
	J. Roth	Chief	D, NIAMDD
	B.V. Howard	Assistant Chief	PCRS, NIAMDD
	D.M. Mott	Research Biochemist	PCRS, NIAMDD
	I. Klimes	Guest Worker	PCRS, NIAMDD
	B. Vasquez	Postdoctoral Fellow	PCRS, NIAMDD

COOPERATING UNITS (if any)
R.H. Unger, Human Diabetes Study Center, Phoenix, AZ; R. Andres, NIA, Baltimore, MD; D. Elahi, NIA, Baltimore, MD; A.H. Rubenstein, Univ. of Chicago, Chicago, IL

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 6.0	PROFESSIONAL: 1.5	OTHER: 4.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Age, sex and weight matched groups of genetically normal, prediabetic and diabetic Pima Indians and normal Caucasians were examined by means of oral and intravenous glucose tolerance tests, arginine infusions, in vivo insulin resistance measurements, glucose clamp technique and circulatory mononuclear cell insulin receptor assays, and urinary protein profiles to determine whether abnormalities of insulin, glucagon secretion or other measurements are characteristic of the prediabetic, diabetic, obese and non-obese states.

Project Description:

This investigation is a continuation and expansion of the previous projects: 1) Evaluation of the Prediabetic and Diabetic State in the Pima Indians (Z01 AM 69003-0 EFSB), 2) Evaluation of the Prediabetic State in the Pima Indians-Caucasian Controls (Z01 AM 69004-0 EFSB), 3) Evaluation of Basement Membrane Thickness in Diabetic Pima Indians (Z01 AM 69005-05 EFSB).

Objectives: Many components of the natural history of diabetes remain to be described. A prospective study of genetically suspect prediabetic subjects (defined as the non-diabetic offspring of two diabetic parents) appears to be the most direct and reliable method of describing the natural history of the disease.

Study of the prediabetic will enable us to better understand the interrelationship of glucose levels with insulin and glucagon levels, an area which has remained controversial. These studies will also clarify the relationship of hyperglycemia to microangiopathy. Detailed studies of urinary protein abnormalities and muscle capillary basement membrane thickness have been examined in the Pima and should clarify the history of some of the earliest physical abnormalities found in diabetes.

Obesity associated with hyperinsulinemia is a significant factor in the development of diabetes in this population. Therefore, a prospective study of the obese and pre-obese subjects could help in understanding the factors related to the eventual development of diabetes in the Pima Indians.

The purposes of the study are:

1. To determine whether there are abnormalities in carbohydrate metabolism gluco-regulatory hormone secretion, urinary protein excretion or capillary basement membrane thickening in nondiabetic Pima Indians compared with nondiabetic Caucasian controls.

2. To determine if there are differences in these variables between Pima Indians with two diabetic parents and those with two nondiabetic parents.

3. To determine the differences in carbohydrate metabolism and glucoregulatory hormone secretion between lean and obese Pima Indians.

4. To initiate a long-term prospective study of prediabetes.

Methods Employed: Obese and lean Pima Indians, with normal glucose tolerance, nondiabetic offspring of two diabetic parents, nondiabetic offspring of two nondiabetic parents, subjects with long and short duration of documented diabetes and nondiabetic Caucasians with two nondiabetic parents were studied.

Following dietary proparation all subjects were admitted to the Clinical Research Center for testing. Tests included an oral glucose tolerance test, an intravenous glucose tolerance test, an arginine infusion, fundus photographs and 24-hour urine collected for determination of specific urinary proteins by nephelometry.

Two sub-groups of diabetics were examined; a) long-term diabetics, defined as subjects with fasting plasma glucose ≥140 mg/100ml or two hour post-carbohydrate load glucose > 300 mg/100ml and > 10 years duration with and without retinopathy, and b) short-term diabetics defined as subjects who have had a documented marked deterioration in their oral glucose tolerance over the last five years from a two hour post-carbohydrate load plasma glucose ≤140 mg/100ml to a two hour plasma glucose ≥300 mg/100ml.

The very high prevalence of diabetes in the Pima people, while providing a very large group of genetically prediabetic individuals, raised a problem of finding individuals who are truly normal. Genetically normal Pima offspring were selected from phenotypically normal parents 45 years of age or older. It is possible, however, that with the high prevalence of diabetes in this tribe, virtually all the Pimas could carry the diabetic genome. Because of this possibility, a group of non-diabetic Caucasians with nondiabetic family histories were examined as an additional control group to compare with the "normal" Pimas.

Studies of insulin secretion and insulin action were performed on Pima Indians and Caucasians over a range of obesity. Tests of insulin action included the measurement of insulin resistance, a) using an infusion of insulin, glucose and somatostatin and b) glucose clamp of technique of Andres.

Tests of insulin secretion included the oral glucose tolerance test, intravenous glucose tolerance test, arginine infusion and standard meal. Insulin secretion during hyperinsulinemia was determined using the "euglycemic clamp" technique of Andres.

Results:

1. Insulin Levels in the Pima. The pattern of insulin response to oral and intravenous glucose has been claimed to be characteristic of diabetes and prediabetes. While insulin responses to oral glucose were reduced in subjects with two hour glucose levels over 225 mg/dl and the acute phase insulin release was absent in all diabetics there was evidence of hyperinsulinemia in the Pima nondiabetics compared to the Caucasians. The normal and prediabetic Indians had higher stimulated insulin levels during all the tests than those on the Caucasians. Differences in insulin levels between the two could not be explained by differences in glucose level, age or obesity. These higher plasma insulin levels in the Pima may be because of a primary abnormality in insulin secretion due to a hypersecretory β cell or occur as a secondary phenomenon to compensate for increased *in vivo* insulin resistance.

2. C-Peptide Studies. To assess whether hepatic insulin extraction is diminished in the Pima, serum insulin and C-peptide concentrations were determined during oral glucose tolerance tests performed on ten Pima Indians and ten Caucasians with normal glucose tolerance and a range of fasting insulin levels. The results indicate that there is no abnormality of hepatic insulin extraction in the Pimas. In addition, this study suggests that the fractional hepatic extraction of insulin does not change over a wide range of obesity and insulin levels.

3. Studies of Insulin Resistance. An improved method has been developed to measure *in vivo* resistance to insulin mediated glucose disposal in order to investigate the mechanism of the hyperinsulinemia and diabetes prevalent in the Pima Indians. A mixture of glucose (420 mg/min), insulin (0.77 mu/kg/min), and somatostatin (500 µg/hr) was infused for 150 minutes. A steady state level of plasma insulin (SSPI) and glucose (SSPG) was attained after 90 minutes. Endogenous insulin secretion determined by C-peptide measurement and glucagon remained suppressed throughout the infusion. The above method was used to determine whether insulin resistance of obese glucose intolerant subjects can be accounted for by obesity alone. Fourteen obese glucose intolerant and twenty-one diabetic Southwestern American Indians with similar degrees of obesity were studied. The mean SSPG was higher in the glucose intolerant subjects than in weight matched subjects with normal glucose tolerance (226±10 vs 136±13 mg/dl $p<0.0001$) indicating that obesity was not the only factor responsible for the increased resistance in obese glucose intolerant subjects. This increased resistance to insulin action was found in the presence of similar insulin binding to mononuclear cells. It appears that in obese Southwestern American Indians with glucose intolerance, abnormalities beyond the site of insulin binding to the receptor may explain the observed increase in *in vivo* insulin resistance.

To determine whether the Pima Indians with normal glucose tolerance have increased *in vivo* resistance compared to weight matched Caucasian subjects, twenty-two Pima Indians and twenty Caucasians were studied. The SSPG levels increased with increasing obesity (BMI) in both Pima Indians ($r=0.57$) and Caucasians ($r=0.47$). Adjusting for age, sex, and BMI the SSPG averaged 28±13 mg higher in the Indians than in the Caucasians. Insulin binding to mononuclear cells was measured in a group of Pima and Caucasians and found to be similar.

Our studies show that the Pima Indians have hyperinsulinemia and higher *in vivo* insulin resistance than Caucasians with equivalent degree of obesity and glucose tolerance. The role of hyperinsulinemia and of insulin resistance in the pathogenesis of obesity and diabetes in the Pima remain to be defined. Furthermore, it is unclear whether insulin resistance appears first or if it is secondary to hyperinsulinemia. Only longitudinal studies of these factors will elucidate their role in the development of diabetes and obesity.

4. Studies of Islet Cell Function During Hyperinsulinemia and Euglycemia. Several groups have demonstrated that exogenous insulin inhibits endogenous insulin secretion in normal subjects. If the negative feedback constitutes an important physiological control over insulin secretion then the hyperinsulinemia of the obese would conceivably involve the defect of this regulatory system. A similar defect in regulation of insulin secretion could also partly explain the hyperinsulinemia observed in Pimas as compared to Caucasians. The present study was therefore designed to examine the β cell (and α and PP cell) response to hyperinsulinemia at physiological levels (100 μ/ml above basal) in groups of obese and nonobese Pima Indians and Caucasians using the euglycemic clamp technique.

Our studies demonstrate that C peptide levels fall in response to hyperinsulinemia and that the effect is seen in obese as well as in lean subjects. The magnitude of the C peptide drop was larger in the obese than in the lean, but the percentage fall was similar. During hyperinsulinemia, the suppressed CPR levels in the obese were still much higher than either the insulin-suppressed levels in the obese or the basal levels in the lean. Plasma glucagon levels were comparably suppressed by insulin in lean and obese subjects. The rates of recovery from insulin suppression differed in the two types of islet cells. The β cell recovered more rapidly than the alpha cells.

This study presents evidence to suggest that insulin in physiological amounts can inhibit both the α and β cell and that in the obese complete resistance of the β cell to insulin can be excluded. It is therefore possible that the hypersecretion of insulin in obese subjects could be due to (1) increased β cell mass with normal suppressibility by insulin or (2) that there is subpopulation of β cells in the islets of obese subjects which is hypersecretory and resistant to regulation by insulin.

Ten lean and ten obese Caucasians and ten obese Pima-Papago Indians were studied. None were diabetic. Plasma insulin was raised acutely and maintained for 90 minutes; euglycemia was maintained by the clamp method during hyperinsulinemia and during the 90-150 minute recovery period. Base line C-peptide levels in the two obese groups averaged 2-4 times those of the lean subjects. During hyperinsulinemia, a gradual decline of C-peptide occurred in all groups. The suppressed CPR levels in the two obese groups averaged 0.77 pm/ml and 0.82 pm/ml, about 2-2.5 times as high as the basal levels in the lean (0.33), and were 4-5 times higher than the suppressed levels in the lean group (0.17 pm/ml). Plasma glucagon levels in all groups were suppressed to the same extent during and after the insulin infusion. To further investigate the causes of hyperinsulinemia in obesity, a study of six obese and four lean Pima Indians has been carried out. On three different days plasma insulin was raised acutely 10, 25 or 100 μU/ml above the fasting concentration and maintained for 90 minutes. In the pre-clamp period insulin and C-peptide levels of the obese subjects were three fold greater than those of the lean. On preliminary analysis of the data, we find that the maximum suppression of C-peptide levels occurred at the highest plasma insulin levels (100 μU/ml above basal) and the C-peptide suppression was similar in both obese and lean groups. Plasma glucagon was also suppressed to a comparable degree in both groups.

5. Urinary Protein Excretion in Diabetics. This study was designed to determine if good control of plasma glucose levels in obese Type II diabetic subjects could result in a reduction of urinary protein excretion. Twenty-four hour urine samples were collected before and after hypocaloric diet therapy for a 2-4 month period in seven nondiabetic, seven glucose intolerant and twenty-seven diabetic subjects. After diet therpy, fasting and two hour plasma glucose levels after oral glucose load remained normal in nondiabetics and improved significantly in both the glucose intolerant and diabetic subjects. The concentration of plasma proteins and albumin present in the urine were measured by an immunonephelometric technique using specific antisera against human total plasma protein and albumin. No changes were found in either total protein or albumin in the nondiabetics. Protein concentrations decreased in both the intolerant and the diabetic subjects. In two cases diminished proteinuria has persisted from eight to ten months. The findings suggest that control of hyperglycemia is associated with reduction of proteinuria in Type II diabetics.

Significance to Biomedical Research and the Program of the Institute: These studies are providing information on issues which have been controversial in diabetes research for over a decade. They are making it possible to systematically approach the cause of diabetes in the Pima, a group with the highest known prevalence of maturity onset diabetes.

Proposed Course: Subjects will be recalled every two years for an additional series of tests. The second recall is now in progress. This should enable careful documentation of the natural history of diabetes. Additional studies will be conducted to evaluate the site of insulin resistance in the hyperinsulinemic Pimas. Further studies of islet cell function are also planned to determine the factors which could cause the hyperinsulinemia in the Pima Indian. Longitudinal studies of these factors may explain their role in the development of diabetes and obesity.

Publications:

1. Nagulesparan, M., Savage, P.J., Mott, D.M., Johnson, G.C., Unger, R.H., and Bennett, P.H.: Increased in vivo resistance in obese, glucose intolerant southwestern American Indians. Evidence for a defect not explained by obesity. J. Clin Endocrinol. Metab. 51: 739-743, 1980.

2. Aronoff, S.L., Schnider, S., Smeltzer, J., MacKay, W., Tchou, P., Rushforth, N., Miller, M., and Bennett, P.H.: Urinary excretion and renal clearance of specific plasma proteins in diabetes of short and long duration. Diabetes, in press.

NIH Contract No. N01-AM-6-2219

Starting Date: September 1, 1980
Expiration Date: October 31, 1981

Contractor: University of Texas Health Science Center
Southwestern Medical School

Money Allocated: $2,794,619.

Man years Purchased: 32.2

This contract is for extension of the studies carried out under the NIH Project Z01 AM 54018-0 , Characterization of Metabolic and Morphologic Abnormalities in Diabetes.

Work to date has been comprised of consultation and collaboration on all studies of diabetes, obesity and lipid metabolism and the measurement of glucagon, insulin, lipids and lipoproteins on samples obtained by NIH personnel and three contract physicians at the Phoenix Clinical Research Center. All studies underway utilize contract personnel working both in Phoenix and in Dallas.

Proposed Course:

The collaboration on all studies in progress at the Phoenix Clinical Research Center will continue as planned.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54019-05 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cellular Metabolism in Diabetic and Nondiabetic Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.V. Howard	Assistant Chief	PCRS, NIAMDD
OTHER:	D.M. Mott	Research Biochemist	PCRS, NIAMDD
	F. Ishibashi	Visiting Scientist	PCRS, NIAMDD
	B. Vasquez	Postdoctoral Fellow	PCRS, NIAMDD

COOPERATING UNITS (if any) J. Larner, Dept. of Pharmacology, Univ. of Vermont, School of Medicine; Human Diabetes Study Center, Univ of TX Southwestern Medical School Dallas; H. Hidaka, Univ. of Osaka, School of Medicine, Japan

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Elevated insulin levels in Pima Indian adults compared to weight matched Caucasian controls suggest that cellular changes external to the pancreas may be important in the pathogenesis of diabetes mellitus. The aim of this project is to study several aspects of insulin function in cells obtained from normal and diabetic Pima Indians, in order to determine if diabetes mellitus is expressed in genetic differences at the cellular level. Fibroblast cultures have been established from normal and diabetic Pima Indians. Parameters of cell growth are being compared in the normal and diabetic cells, and insulin binding is being studied. In addition, cellular metabolism and transport functions are being studied in the presence and absence of insulin, in order to further investigate cell insulin action, to determine if cellular abnormalities in insulin response occur in individuals with diabetes and to investigate factors which may induce cell insulin resistance.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Resistance to insulin mediated glucose uptake is a well documented component of the obesity and diabetes which are so prevalent in the Pima population. Diploid fibroblast cultures established from skin biopsies afford an easily accessible system for the examination of cellular metabolic capacity which is isolated from physiological variables. This project has dual objectives: (1) to examine the action of insulin on glucose metabolism in the fibroblast. Several aspects of glucose metabolism including hexose transport, glycogen synthesis, and CO^2 production will be examined and the mechanism of insulin action on these parameters will be investigated. (2) to establish cultures from biopsies of genetic normal, prediabetic and diabetic Pima Indians and age-matched Caucasians controls and to compare sensitivity and magnitude of response to insulin using the methods developed in (1).

Major Findings:

(1) Investigations of insulin action in the cultured fibroblast.

(a) Insulin binding. The fibroblast has been shown to possess a specific insulin receptor which is regulated by physiological levels of insulin. Results from studies of whole cell receptor binding and insulin and MSA binding to partially purified insulin receptor preparations from the fibroblast are summarized in Z01 AM 54020-03 DD.

(b) Insulin stimulation of hexose transport. Insulin stimulation of 2-deoxyglucose uptake in cultured human skin fibroblasts has been examined in the presence of varying concentrations of extracellular glucose. When cell monolayers were preincubated in glucose free medium, insulin stimulation of hexose transport was small. Increasing glucose levels from 2.7 - 16.7 mM in the preincubation medium enhanced insulin stimulated 2-deoxyglucose uptake (18-65% of basal uptake). Insulin stimulation of hexose transport in the presence of glucose was the result of an increase in Vmax without significant change in the Km of transport. Maximum enhancement of insulin action was achieved 18-24 hours after glucose exposure. After 24 hours insulin response decreased. Glucose did not influence the concentration of insulin required for half maximal stimulation. Growth conditions also altered the magnitude of insulin stimulation of 2-deoxyglucose uptake; response was maximal in confluent cells (5-10 days of culture) and decreased during senescence. These data demonstrate that extracellular glucose levels influence insulin action in cultured fibroblasts. This implies that insulin responsiveness in vivo may be regulated in some systems by ambient glucose levels.

(c) Insulin stimulation of glycogen synthase. The effect of insulin on glycogen synthase activity in human diploid fibroblasts has been studied. As little as 2×10^{-10}M insulin increased the glycogen synthase I activity without changing the total activity. Stimulation occurred within 5 minutes and became maximal in 30 minutes. A half maximal increase of I activity was achieved at 3×10^{-9}M insulin. Glucose starvation increased the magnitude of response of glycogen synthase to insulin but did not change the insulin concentration necessary to give a half maximal stimulation. Glucose increased the basal level of I activity in human fibroblasts; the effect of insulin was additive. During in vitro senescence the total glycogen synthase activity declined, but the concentration of insulin which produced a half maximal stimulation remained unchanged.

(d) Insulin action in glucose oxidation. Insulin action on glucose oxidation has been examined in human diploid fibroblastic cells. When the fibroblasts were preincubated in the absence of glucose, insulin stimulation of ^{14}C-6-D-glucose oxidation was small (< 20%). Preincubation in glucose containing medium (2.7 - 11.0 mM) for 18 hours enhanced insulin stimulation of ^{14}C-6-D-glucose oxidation to a maximum of 240% of control. Stimulation was observed with as little as 10^{-10}M insulin and reached maximum at 10^{-8}M insulin. Half maximum stimulation was achieved at approximately 9 X 10^{-10}M insulin at all glucose concentrations. In order to examine the specificity of insulin action on glucose oxidation, the relative effectiveness of various insulin analogues was examined. The order of potency was porcine insulin (100%)> proinsulin (70%)> bonito insulin (54%)> desoctapeptide insulin (37%). Multiplication stimulating activity (MSA) also stimulated glucose oxidation with a half maximal stimulation at 3 X 10^{-9}M. The optimal pH for insulin stimulation of glucose oxidation was 7.4, and insulin action was less at temperatures lower than 37°C. When the influence of growth conditions on insulin stimulation of glucose oxidation was assessed, confluent cells had greater responsiveness than dividing cells, and responsiveness declined in senescent cells. When insulin action and the effects of glucose preincubation were examined using ^{14}C-1-D-glucose, insulin action after glucose deprivation was again less than 20%, but glucose preincubation had only a small enhancement of ^{14}C-1-D-glucose oxidation (40%). To investigate the metabolic site of insulin action and glucose enhancement of glucose oxidation, the effect of insulin on the distribution of incorporated ^{14}C-glucose was compared in cells preincubated in the presence or absence of glucose. Insulin stimulation of lactate production (glycolysis) was significantly enhanced by glucose preincubation, whereas glucose incorporation into ribose and glycogen was reduced. Thus, insulin action on CO_2 production seemed to be a reflection of insulin action at the site of glycolytic-TCA pathways; the preferential glucose enhancement of ^{14}C-6-D-glucose oxidation appeared to be due to the increased porportion of glucose used for glycolysis and also to an enhancement of insulin action in the glycolytic pathway. These studies indicate that under appropriate culture conditions insulin significantly stimulates ^{14}C-6-D- glucose oxidation in cultured human skin fibroblasts. Since insulin action in the fibroblasts appears to have similar fundamental characteristics compared to insulin action in other cell types, this might be a useful system for further study of insulin action on glucose metabolism.

(e) Influence of pH on insulin action. In order to elucidate the mechanism of decreased insulin action observed during diabetic ketoacidosis, the influence of pH and 3-hydroxybutyrate (3OHBA) on insulin binding and action has been studied in cultured human diploid fibroblasts. When the pH of the incubation medium was decreased from 7.6 to 6.8, insulin stimulation of glycogen synthase and total glucose uptake were decreased. The decreased pH caused both an increase in the insulin concentration necessary for half maximal response, and a decrease in maximal responsiveness. When insuin binding was measured at lower pH, receptor affinity was decreased. The effect of 3OHBA on insulin binding and action was assessed at pH 7.4 and 6.9. In the presence of 3OHBA, insulin binding increased but insulin stimulation of glycogen synthase was unaffected. The data show that insulin action is impaired at lower pH. The decreased sensitivity is probably related to the decrease in insulin binding affinity at lower pH, but decreased maximal responsiveness implies that cellular post-receptor components are also affected by lower pH. The results indicate that in diabetic ketoacidosis the decreased pH rather than 3OHBA is the primary mediator on interference in insulin action.

(f) Energy requirements for insulin action - The effect of adenosine. The effect of adenosine on insulin stimulation of 2-deoxyglucose uptake in human diploid fibroblastic cells has been studied in order to examine factors which influence insulin action on hexose transport in this system. Preincubation with adenosine for 18 hours enhanced insulin stimulation of 2-deoxyglucose uptake. Adenosine appeared to act in concert with glucose with maximum enhancement at 1.25 mM adenosine and 5.4 mM glucose. Adenosine did not influence insulin action on glucose oxidation. Adenosine acted by increasing the maximum stimulation (> 100% increased over basal uptake) without influencing the insulin concentration for half maximal stimulation (10^{-9} M). Insulin stimulation of 2-deoxyglucose uptake in the presence of adenosine resulted from an increase in Vmax without significant change in the Km of transport. The time required for maximal insulin action in the presence of adenosine was 60 minutes, which was similar to that obtained for other parameters of insulin action in fibroblasts. Various deoxyadenosine analogues did not influence insulin stimulation of 2-deoxyglucose uptake, suggesting that adenosine was not acting via adenylate cyclase. Neither guanosine nor inosine influenced insulin action, indicating metabolism of adenosine (i.e. ribose) was not the mechanism of action. The inhibiton of adenosine deamination did not influence adenosine enhancement of insulin action. On the other hand, 2,4-dinitrophenol and KCN reversibly inhibited adenosine enhancement of insulin action. When distribution of ^{3}H-adenosine was examined, it was incorporated mainly into ATP, and ATP formation increased with increasing glucose. These data demonstrate that (1) adenosine enhances insulin action on hexose transport in fibroblasts, (2) adenosine appears to effect hexose transport by increasing available energy supply, and (3) insulin action on 2-deoxyglucose transport in the fibroblast is dependent on availability of ATP.

(g) Cell space measurements in cultured human diploid fibroblasts in monolayers or in suspension. Cultured human diploid fibroblasts are a useful system for a variety of metabolic studies for which measurement of cell space is often necessary. Markers commonly used for measurement of total water space (TWS) and extracellular space (ECS) were investigated for use in the determination of intracellular space (ICS = TWS - ECS). Methods for determination of cell space of fibroblasts both in monolayers (FM) and suspension (FS) were compared. Incubation of markers (0.1 mM, 1-5 μC_i/ml) at 37° with FS were terminated by centrifugation through oil (2:1, dibutyl phtalate: dinonyl phthalate). FM incubations were terminated by alternately aspirating and rapidly rinsing the plates 3 times with buffer at 4°. The space (μl/10^6 cells, x ± SEM) available to the TWS marker, [^{14}C]-urea, plateaued at 10-15 min in both FM (3.04 ± 0.06) and FS (2.20 ± 0.05) and was independent of marker concentration (0.05-1.00 mM). The slow rate of equilibration of [^{14}C]-urea (relative to ^3H_2O)is an advantage for a TWS marker for FM, because loss of TWS marker from IC during the rinse step is minimized. The time course of distribution of ECS markers with FS showed [^{3}H]-sucrose (0.21 ± 0.02) and [^{3}H]-insulin (0.25 ± 0.03) spaces remained constant over 30 min. Both [^{14}C]-L-gluocse and [^{3}H]-D-mannitol increased with time (to 0.54±0.05) and 0.79 ± 0.08 at 30 min) suggesting penetration to the ICS. Double label experiments yielded ICS values (20 min urea space minus 5 min sucrose space) of 2.57 ± 0.06 for FM and 2.03 ± 0.05 for FS.

(h) Action of alloxan on glucose metabolism. The influence of alloxan on the uptake and metabolism of glucose in mammalian cells has been investigated using human diploid fibroblastic cells in culture, in order to elucidate the relation between alloxan action and insulin secretion in the β-cell. When monolayers of cells were exposed to radiolabelled glucose, the presence of alloxan (0.31-1.87 mM) resulted initially in a dose dependent enhancement of total cell glucose incorporation. This was observed within 2 minutes and declined by 6 minutes. After that time, alloxan inhibited glucose incorporation. When hexose transport was examined directly using glucose analogues, alloxan neither enhanced, nor inhibited the kinetics of uptake of 3-O methyl-D-glucose or 2-deoxy-D-glucose. Alloxan (0.62-1.87 mM) exerted no measurable effect on cell permeability as determined by cell volume measurements using sucrose and D-mannitol. Thus alloxan appears to directly influence cell glucose metabolism beyond the level of phosphorylation. Alloxanic acid (0.62 - 1.87 mM), a decomposition production of alloxan, did not affect glucose incorporation in the fibroblasts. In addition, prior exposure of fibroblasts to alloxan (0.62±1.87mM) for six minutes did not influence trypan blue exclusion by fibroblasts, nor impair ^{3}H-leucine incorporation in the fibroblasts. Analysis of cell extracts after cell monolayers were exposed to ^{14}C-U-D-glucose indicated that the initial stimulation by alloxan was observed primarily in the nucleotide fraction (ribose) with inhibition of lactate production. The subsequent inhibition was observed in the nucleotide fraction. Assay of $^{14}CO_2$ production indicated that alloxan enhanced $^{14}CO_2$ formation from ^{14}C-U-D-glucose for approximately 10 minutes followed by inhibition. In order to probe the site of alloxan action, rates of $^{14}CO_2$ formation from 1- and 6 position labelled ^{14}C-glucose were compared. The initial stimulation was observed mainly in ^{14}C-1-D-glucose oxidation, whereas inhibition was measurable with the 6-positioned tracer. The results suggest that alloxan initially stimulates the pentose-monophosphate shunt and the glycotic-TCA pathway.

(i) Alloxan and H_2O_2 action on glucose metabolism in cultured fibroblasts: oxygen-containing free radicals as a mechanism of alloxan action. In order to elucidate the mechanism of alloxan action on glucose metabolism the effects of alloxan and H_2O_2 on glucose metabolism in cultured fibroblasts have been compared. Both alloxan and H_2O_2 initially stimulated and then inhibited total cell glucose incorporation. H_2O_2 initially inhibited hexose transport, as measured by 2-deoxy-glucose uptake, while alloxan had no effect on transport. The distribution of incorporated ^{14}C-U-D-glucose in the presence or absence of alloxan and H_2O_2 was examined chromatographically. Both agents initially enhanced nucleotide formation followed by an inhibition. Alloxan initially inhibited and then enhanced glucose conversion to lactate, while H_2O_2 had opposite actions. The effects of alloxan and H_2O_2 were investigated using differently labelled ^{14}C-glucoses. Both agents caused an initial stimulation and then a subsequent inhibition of oxidation of ^{14}C-U-D- and ^{14}C-1-D-glucose. Alloxan initially stimulated and then inhibited ^{14}C-6-D-glucose oxidation, whereas H_2O_2 initially enhanced it. Catalase abolished all effects of alloxan and H_2O_2 on ^{14}C-U-D-glucose oxidation. Superoxide dismutase and diethylenetriamepentacetic acid, which decreases levels of O_2- and OH-, prevented the delayed inhibitory actions of alloxan. These results indicate that (1) the initial action of alloxan is a stimulation of the pentose-monophosphate shunt by the generation of H_2O_2. (2) The subsequent action of alloxan is an inhibition of the glycolytic pathway and pentose-monophosphate shunt by the generation of OH- from O_2- and H_2O_2.

(2) Comparison of insulin action in fibroblasts from diabetic and nondiabetic Pima and Caucasian controls.

(a) Insulin action on glucose metabolism in fibroblasts from diabetics and nondiabetics. To determine if inherent cellular differences in insulin sensitivity account for the insulin resistance of non-insulin dependent diabetes, the effect of insulin on several aspects of cell glucose metabolism was compared in fibroblasts from diabetics and matched nondiabetic controls. The response of total cell glucose metabolism to insulin was assessed by measurement of ^{14}C glucose uptake. Insulin stimulated cell glucose incorporation in nondiabetic cells up to two fold with half maximal stimulation at approximately $3 \times 10^{-9}M$ insulin. This was similar to that observed in diabetic cells. Insulin stimulation of I glycogen synthase activity was also compared in the cells from diabetics and nondiabetics. Both groups demonstrated a three fold increase in %I activity in the presence of insulin with half maximal stimulation at approximately $3 \times 10^{-9}M$. There were no differences between diabetics and nondiabetics in either magnitude of response or insulin concentration for half maximal stimulation. Finally insulin stimulation of hexose transport was compared in the two cell types using 2-deoxyglucose. In both groups hexose transport was elevated approximately 40% over baseline in the fibroblast in the presence of insulin, with half maximal stimulation at approximately $2 \times 10^{-9}M$ insulin. No differences were found in insulin action on glucose metabolism in fibroblasts from diabetics and nondiabetics; these results may indicate that there are no inherent differences in cell sensitivity to insulin's glucoregulatory action in non-insulin dependent diabetics.

(b) Studies of Glucose Metabolism and Insulin Action in Cultures from a Patient with Lipoatrophic Diabetes. Insulin binding and action of insulin on aspects of glucose metabolism have been investigated in cells from a patient with congenital lipoatrophic diabetes and compared to cultures from 9 nondiabetic controls. Incorporation of glucose was elevated in the patient's cells at glucose levels above 0.1 mM. When distribution of labelled glucose was examined, cell associated glycogen and acid soluble material were increased, but the greatest increment was in lactate production. Insulin binding, as indicated by maximum specific ^{125}I-insulin binding and concentration of unlabelled insulin for 50% displacement, was normal, although insulin regulation of the insulin receptor was less. The cells from the patient had reduced response of total glucose incorporation to insulin. When insulin stimulation of glycogen synthase was measured directly, the response to insulin was also attenuated. On the other hand, hexose transport levels and insulin stimulation of hexose transport appeared to be unimpaired. The data indicate alterations in both cell glucose metabolism and in response to insulin which may be related to the observed insulin resistance of this disorder.

Significance to biomedical research and the program of the institute: Resistance to insulin mediated glucose uptake is a well documented component of obesity and diabetes. It is not possible from *in vivo* studies, even if accompanied by measurements of freshly collected blood cells or tissues, to determine if the observed insulin resistance is a secondary response to the other dynamics of the hyperglycemic or obese state, or if it is a reflection of inherent cellular differences in response to insulin which contributed to the development of these disorders. Diploid fibroblast cultures established from skin biopsies afford an easly accessible system for the examination of human cellular metabolic capacity which is isolated from physiological variables. Our data to date indicate that the human fibroblast possesses a specific insulin receptor and that several points of carbohydrate metabolism respond to physiologic levels of insulin. The results demonstrate that this method is a powerful tool for the comparison of insulin sensitivity of cells derived from patients with disorders of insulin action. The availability of the Pima population is a further advantage. Extensive clinical and demographic data on several generations are availabel for selection of subjects, and the homogeneity of the population is an advantage in eliminating other variables which might confound the comparison. The results to date suggest the impaired insulin sensitivity observed in *in vivo* studies of individuals with abnormal glucose tolerance may not be due to inherent genetic differences in sensitivity to insulin action but rather to the complex physiological changes associated with the hyperglycemic state. Future studies combining measurements of insulin binding, receptor regulation, hexose transport and other intracellular responses to insulin, will lead to an understanding of the mechanism of insulin resistance and its cellular basis.

Proposed Course:

1. Studies of cell insulin action. We will continue to examine insulin stimulation of glucose metabolism in the fibroblast, in order to further investigate the mechanism of insulin action on cell metabolism and to investigate variables which influence the cell response to insulin. These studies will include:

(a) Correlation of insulin binding and action. Insulin binding will be measured on monolayer cells and correlated with insulin action on glycogen synthase and total cell glucose uptake. Effect of down regulation of the insulin receptor on insulin action will also be examined. This will yield data on insulin action when insulin binding and response are measured under identical conditions.

(b) Examination of the action of insulin analogs and mitogens on cell glucose metabolism. Insulin analogs and mitogens such as MSA will be compared to their actions on the parameters of glucose metabolism examined to date. The data will be correlated with binding data obtained in whole cell and isolated receptor fractions (see Z01 AM 54020-02-DD). Cell cultures from the patient with lipoatrophic diabetes will be included in these studies in order to further examine the attenuated response observed in these cells.

(c) Examination of an intracellular intermediate of insulin action in the fibroblast. In coordination with Dr. Larner extracts of insulin stimulated cells will be assayed for the presence of a low molecular weight intermediate of insulin action observed previously in muscle and adipocytes. If present the chemical properties of the intermediate will be examined and the system will be used for study of time course and long term metabolism of this compound.

2. Comparison of diabetic and nondiabetic fibroblasts. Since no inherent differences in insulin action on glucose metabolism has been detected in diabetic cells, studies will center around the hypothesis that insulin resistance is induced by the hyperglycemia, hyperinsulinemia or other changes induced in the diabetic state. Therefore influence of glucose, insulin and other counterregulatory hormones will be examined on cell insulin action using the systems described above. In addition, since the fibroblast is not a quantitatively significant target tissue for *in vivo* insulin action, studies will be extended to the endothelium. The latter constitutes an important first site of plasma insulin action *in vivo*. Endothelium may be isolated from human sources (umbilical veins) and cultured in vitro. The systems for assay of insulin action which were developed for the fibroblast will be employed for examination of insulin action in this cell type. In addition, the availability of umbilical cords from diabetic mothers will allow comparative studies of diabetic and nondiabetic endothelium.

Publications:

1. Hidaka, H., Howard, B.V., Kosmakos, F., Fields, R.M., Craig, J.W., Larner, J.L., and Bennett, P.H.: Insulin stimulation of glycogen synthase in cultured human diploid fibroblasts. Diabetes 29:806-10, 1980.

2. Hara, H., Hidaka, H., Kosmakos, F., Mott, D.M., Howard, B.V., and Bennett, P.H.: Characterization of the human insulin receptor isolated from cultured fibroblasts and erythrocyte cell membranes. J. Clin. Endocrinol. Metab. 52:1-22, 1981.

3. Hidaka, H., Howard, B.V., Ishibashi, F., Kosmakos, F.C., Craig, J.W., Bennett, P.H., and Larner, J.: Effect of pH and 3 hydroxybutyrate on insulin binding and action in cultured human fibroblasts. Diabetes 30:402-6, 1981.

4. Howard, B.V., Mott, D.M., Hidaka, H., Fields, R.M., Katzeff, H., Howard, W.J., and Bennett, P.H.: Cell culture studies of a patient with congenital lipoatrophic diabetes - normal insulin binding with alterations in intracellular glucose metabolism and insulin action. Metabolism, 1981 (in press).

5. Howard, B.V., Hidaka, H., Ishibashi, F., Fields, R.M., and Bennett, P.H.: Type II diabetes and insulin resistance - evidence for lact of inherent cellular defects in insulin sensitivity. Diabetes 1981 (in press).

6. Ishibashi, F., Hidaka, H., Fields, R.M., Howard, B.V., and Bennett, P.H.: Alloxan action on glucose metabolism - cultured fibroblasts I stimulation and inhibitor of glucose utilization. Am. J. Physiol. 1981 (in press).

7. Ishibashi, F., Bennett, P.H. and Howard, B.V.: Alloxan action in glucose metabolism in cultured fibroblasts II effects in pentose-monophosphate shunt and tricarboxylic acid pathways. Am. J. Physiol. 1981 (in press).

8. Ishibashi, F., and Howard, B.V.: Alloxan and H_2O_2 Action on glucose metabolism in cultured fibroblasts - generation of oxygen containing residuals as a mechanism of alloxan action. J. Biol. Chem. 1981 (in press).

9. Ishibashi, F,. Hidaka, H., and Howard, B.V.: Glucose enhancement of insulin action - elevated glucose levels increase insulin stimulation of 2-deoxyglucose uptake in in cultured human fibroblasts. J. Clin. Endocrinol. Metab. 1981 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54020-04 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of Insulin Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.M. Mott	Research Biochemist	PCRS, NIAMDD
OTHER:	B.V. Howard	Assistant Chief	PCRS, NIAMDD
	M. Nagulesparan	Guest Worker	PCRS, NIAMDD
	B. Vasquez	NIH Postdoctoral Fellow	PCRS, NIAMDD
	P.H. Bennett	Chief	EFSB, NIAMDD

COOPERATING UNITS (if any)
Human Diabetes Study Center, University of Texas Health Science Center

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 2.9	PROFESSIONAL: 0.9	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Regulation of insulin receptors is being studied in vivo using circulating mononuclear cells and in vitro using cultured diploid fibroblasts. Down regulation in the fibroblast has been used to detect inhibitors of insulin action in plasma from insulin resistant subjects. Investigation of monocytes and fibroblasts suggest that cells from obese subjects have more receptors, reduced insulin affinity, increased insulin degradation and decreased down regulation. Insulin binding to monocytes from obese glucose tolerant and intolerant subjects was the same, indicating that a source of insulin resistance distinct from the receptor is present in obese Pima subjects. Insulin receptors have been solubilized and partially purified from cultured human diploid fibroblasts and from human erythrocytes. Characterization of insulin binding to solubilized insulin receptor preparations resulted in binding affinity, pH optimum and analog specificity data similar to those for the intact cells. The binding of Multiplication Stimulating Activity (MSA) as well as insulin were measured in suspended human fibroblasts and in solubilized receptor preparations. Sephadex G-200 chromatography did not separate the two receptors, although characterization of the specificity of binding clearly predicts two separate binding sites.

Project Description:

Objectives: Our objectives include (1) to determine if the increased obesity of the Pima subjects is related to changes in kinetics of insulin binding or in receptor regulation, (2) to use the cultured fibroblast to investigate genetic differences in insulin binding, degradation and receptor regulation between diabetic and normal Pima and Caucasian subjects, (3) to use solubilized fibroblast insulin receptor preparations to examine the structure and regulation of the insulin receptor and its relation to mitogen binding, (4) to study plasma from obese and diabetic subjects for their effect on in vitro insulin receptor regulation. This last technique will be used to detect possible inhibitors of insulin action in plasma from insulin resistant subjects.

Results:

1. Insulin binding to Pima and Caucasian Monocytes. ^{125}I-insulin binding was studied using circulating mononuclear cells from 19 normal Pima and 18 normal Caucasian subjects over a wide range of obesity. Both groups showed a weak negative correlation of specific insulin binding with fasting insulin, with no evidence of differences between Pima and Caucasian groups. Body mass index of the Pima subjects was correlated with the concentration of insulin required for half-maximum displacement of bound ^{125}I-insulin and with the high affinity K_D ($r=0.57$, $p<0.02$ and $r=0.73$, $p<0.0005$ respectively). Similar correlations were obtained for the affinity of Caucasian cells over a similar range of BMI. Because BMI and insulin are so highly correlated in these subjects ($r=0.80$, $p<0.0003$) it is not clear whether insulin or some other factor strongly associated with obesity is responsible for the observed variation in insulin receptor affinity.

The relation between insulin binding and insulin resistance has been studied in two groups of Pima subjects, one with normal glucose tolerance and one with impaired glucose tolerance. Insulin resistance was assessed by measuring insulin-mediated glucose disposal during an infusion of a mixture of insulin, glucose and somatostatin. Despite similar steady state plasma insulin levels, the mean steady state plasma glucose concentration was higher in a glucose intolerant group than in weight-matched subjects with normal glucose tolerance. Insulin binding to mononuclear cells was measured in samples from 8 glucose intolerant [BMI=38±3 (range 30-54), mean fasting insulin 34±3 μU/ml, SSPG=220±11 mg/dl] and 9 normal subjects [BMI=37±3 (range 28-57), mean fasting insulin 24±3 μU/ml, SSPG=148±22 mg/dl]. The percent maximum specific binding per 8×10^6 monocytes per ml was 6.6±0.8 for the glucose intolerant subjects and 7.0±0.5 for the normal subjects (NS). There was no difference in insulin binding curves between the two groups. Thus the decrease in insulin sensitivity in the group with impaired glucose tolerance did not appear to be caused by differences in insulin binding and therefore may be caused by a post-receptor defect in insulin sensitivity.

2. Insulin Binding to Diploid Fibroblasts From Obese Donors. Insulin binding was measured in fibroblasts from 12 nondiabetic subjects with a range of body mass indices (BMI). Group I had BMI <30 and a mean age of 29. Group II had BMI >40 and a mean age of 31. The mean maximum specific ^{125}I-insulin binding was 1.6%/10^6 cells/ml for both groups. However, the ^{125}I-insulin displacement curve for group II was shifted to the right with half maximum displacement (1/2 Bmax) at 18.8±13.8 vs 5.4±3.3 ng/ml. The mean displacement curves were significantly different ($p<0.005$) at insulin levels of 10 and 50 ng/ml. Scatchard analysis indicated that Group II had increased number of receptors (Ro) with decreased affinity. The average affinity profiles demonstrated an elevated limiting high affinity association constant ($\bar{K}e$) for Group I ($55 \mu M^{-1}$ vs $35 \mu M^{-1}$) but the low affinity association constant was similar ($15 \mu M^{-1}$) for both groups. Significant correlations were observed between the cell donor's BMI and 1/2 Bmax ($r=0.78$, $p<0.003$), $1/\bar{K}e$ ($r=0.79$, $p<0.003$) and Ro ($r=0.59$, $p<0.05$). Confluent cell density varied inversely with BMI of the cell donor ($r=0.60$, $p<0.03$). These data suggest that obesity may be associated with genetic differences in insulin binding.

To determine whether the obesity related differences in insulin binding were due to increased insulin degrading activity in fibroblasts from obese subjects, insulin binding and degradation were determined in fibroblasts obtained from six Pima and three Caucasian subjects over a range of BMI. Two different harvest methods were used employing either pretreatment of the culture monolayer with EDTA (0.05%) followed by aspiration, or pretreatment with trypsin (0.05%) plus EDTA (0.02%) and aspiration. The pooled data showed a significant positive correlation between obesity of the donor and insulin degradation ($r=.799$, $p<.05$) by fibroblasts. In addition there were significant differences in insulin binding and degradation between the two harvest procedures. EDTA-harvest caused increased insulin degrading activity (603±156 fmoles x 10^6 cells^{-1} x 4 hr^{-1} vs 91±33 fmoles x 10^6 cells^{-1} x 4 hr^{-1}; $p<.02$), increased number of insulin binding sites (252,000±39,000 vs 114,000 ±29,500 sites/cell, $p<0.001$), increased insulin concentration allowing 1/2 maximal ^{125}I-insulin binding (28.6±4.2 vs 3.37±.14 ng/ml; $p<0.001$), and an increased high affinity K_D ($25.2 \pm 3.3 \times 10^{-10}$M vs $6.16 \pm 0.74 \times 10^{-10}$M; $p<0.001$). Further investigation showed that neither EDTA nor trypsin have a direct effect on insulin degrading activity but that cells harvested with EDTA have a lower viability than trypsin-harvested cells as determined by the uptake of trypan blue. Furthermore, mechanical damage of cells harvested with trypsin altered insulin degradation and insulin binding in a similar manner as harvesting the cells with EDTA. Positive correlations were found between the fraction of stained cells and nonspecific insulin binding ($r=.92$; $p<.0001$), insulin degradation ($r=.78$; p .002), and the high affinity K_D ($r=.80$; $p<.002$). Nonspecific insulin binding was found to correlate with insulin degrading activity ($r=.87$; $p<.0005$) and the high affinity K_D ($r=.79$; $p<.003$). There was also a positive correlation between insulin degrading activity and the high affinity K_D ($r=.89$; $p<.0001$). These results indicate that fibroblasts from obese individuals do exhibit greater insulin degrading activity than cells from lean controls. Furthermore, we have found that the method of harvest and the resulting state of cellular viability markedly affect insulin degrading activity and apparent insulin binding characteristics. Thus previous observations concerning obesity and insulin binding may be explained by differences in insulin degrading activity.

3. In Vitro Insulin Receptor Regulation in Fibroblasts. Maximum insulin mediated reduction in insulin binding (MIR) to diploid fibroblasts from diabetic and nondiabetic Pima Indians and nondiabetic Caucasians was compared for the determination of possible genetic differences in these cells. The mean maximum specific binding per cell was the same in a comparison between 7 diabetic Pimas, 8 normal Pimas and 7 normal Caucasian lines. The mean MIR was also not significantly different for the three groups (58±16% and 64±14%, respectively). When the Pima subjects were subdivided by degree of obesity (i.e. BMI <30 ($n=3$), BMI >39 ($n=5$) the more obese Pima subjects showed a reduced mean MIR (43±14% vs 65±5%).

4. Detection of an Insulin Resistance Related Plasma Factor in Lipoatrophic Diabetes. Plasma from a 32 year old male with congenital lipoatrophic diabetes (LD) with documented severe insulin resistance has been studied for its effects on insulin mediated down regulation of fibroblast insulin receptors. Percent down regulation was calculated from the amount of insulin binding/cell following a 16 hour, 37°C incubation of normal diploid fibroblast monolayers in medium with 20% plasma or 0.1% bovine serum albumin (BSA) and varied insulin concentrations. Maximum down regulation in the presence of LD plasma was significantly reduced compared to the mean obtained using plasma from 4 normal Caucasian subjects (37±6% vs 60±5%, $p<0.001$). When compared to normal plasma over a range of insulin concentrations, LD plasma caused a significant reduction in DR at insulin concentration of ($p<0.002$), 10.0 ng/ml ($p<0.001$) and 100 ng/ml ($p<0.008$). The concentration of insulin required for 1/2 maximal down regulation was increased in the presence of the LD plasma (2.0±0.1 ng/ml for normal vs 8.0±0.8 ng/ml for LD). 20% plasma from normals or LD had no effect on maximum specific insulin binding/cell when compared to preincubation of cells with 0.1% BSA. Normal Caucasian plasma had no significant effect on DR when compared with BSA over the range of insulin concentrations studied. LD plasma did not differ from normals when tested for insulin degradation and did not contain antibodies against insulin or the insulin receptor. Plasma from a diabetic Pima Indian and an obese intolerant Pima Indian had no significant effect on DR when compared with BSA. We conclude that LD plasma contains factor(s) which alter the sensitivity and magnitude of insulin mediated down regulation. In the samples from the Pimas there did not appear to be plasma factors which could contribute to the insulin resistance of this population.

5. Solubilized Insulin Receptor Studies. Insulin receptors have been partially purified from erythrocytes and cultured human diploid fibroblasts. Cell membrane fractions were prepared by differential centrifugation and solubilized by Triton X-100. Gel filtration chromatography on Sephadex G-200 yielded a single peak of insulin binding activity with an apparent molecular weight of 370,000. Solubilized fibroblast receptor preparations showed pH optimum (pH=8.2) and analog specificity (porcine insulin > guinea pig insulin > proinsulin > desoctapeptide insulin) similar to those for whole

cells. Scatchard analysis of insulin binding data for solubilized receptor and intact fibroblasts yielded nearly identical high affinity association constants (Ka) of 1.8 x 10^9M^{-1} and 1.3 x 10^9M^{-1}, respectively. The average affinity profile analysis of the same data yielded limiting high affinity association constants ($\overline{K}e$ of 7.1 x 10^8M and 1.3 x 10^8M, respectively). The solubilized insulin receptor isolated from erythrocytes and fibroblasts displayed very similar parameters of insulin binding and molecular weights. The similarity of the characteristics of the insulin receptor solubilized from these two sources provides evidence against significant modification of the fibroblast insulin receptor by the brief trypsin treatment used to release the cells from the monolayers.

Insulin and mitogens are able to exert similar metabolic effects in target tissues. To clarify this relationship, mitogen and insulin binding were monitored during solubilization and purification procedures using membrane preparations from cultured diploid human fibroblasts. The methods previously used to isolate the membranous fraction of homogenized fibroblasts were modified to exclude receptors originating from intracellular structures. A two-phase polyethylene glycol-dextran centrifugation system was used to purify plasma membranes. The plasma membrane marker, Na^+/K^+ ATPase, was monitored to assess plasma membrane purification. Membrane proteins were solubilized with Triton X-100 and chromatographed on Sephadex G-200. The binding of ^{125}I-insulin and the mitogen, ^{125}I-MSA (Multiplication Stimulating Activity) was compared in suspended fibroblasts and in solubilized receptor preparations. The insulin and MSA receptors copurified and eluted from the G-200 column in the same peak. (Hereafter, the term solubilized receptor indicates the pooled G-200 fractions containing two receptor activities.) Scatchard analysis of insulin binding data yielded nearly identical high affinity association constants (Ka) of 1.7 x 10^9M^{-1} and 1.2 x 10^9M^{-1} for intact cells and solubilized receptor, respectively and the 1/2 Bmax for MSA displacement of insulin were 10nM and 2.7nM, respectively. MSA binding yielded linear scatchard plots with affinity constants (Ka) of 0.10 x 10^9M^{-1} for intact cells and 1.75X x 10^9M^{-1} for solubilized receptor. The increase in affinity for MSA of approximately 10 fold upon purification brings the Ka for MSA into the same range as the high affinity Ka for insulin. In addition, the 1/2 Bmax for insulin displacement of MSA were 30nM and 7.5nM for intact cells and solubilized receptor, respectively. Anti-insulin receptor IgG was able to displace insulin, but not MSA, from intact fibroblasts. The simultaneous purification of two different receptors characterized by different specificities and different binding kinetics (curvilinear vs linear Scatchard plots) are consistent with; 1) separate receptors with very similar molecular weight or 2) the same receptor molecule with separate binding sites for mitogens and insulin.

Significance to Biomedical Research and the Program of the Institute: Our recent results indicate that Caucasian and Pima monocytes in vivo and fibroblasts in vitro respond similarly to insulin to reduce insulin binding, and in both there appears to be a relationship between BMI of the donor and receptor binding. The relationship between BMI and receptor affinity in fibroblasts may be caused by increased insulin degradation by cells from obese subjects. The apparent altered receptor regulation initially observed for hyperinsulinemic Pima subjects thus may be explained by the increased obesity of the Pima compared to the Caucasian subjects. Preliminary results comparing receptor regulation in fibroblasts indicate comparable receptor regulation for normal weight Pima and Caucasian donors, but reduced down regulation of insulin receptors in cells from obese donors. Thus obesity may be associated with genetic differences in insulin binding; these must be further compared.

The results of studies on the lipoatrophic diabetic indicate the presence of plasma factor(s) which inhibits insulin action. In addition, these results demonstrate a technique which can be used to screen plasma from other insulin resistant subjects for factors which alter insulin action.

The methods developed to solubilize and partially purify insulin and mitogen receptors from fibroblasts provide tools for the coordinated study of receptor biochemistry both insulin and MSA and will help clarify the understanding of _in vivo_ action of these peptides.

Proposed Course:

Solubilized Receptor Studies. The simultaneous solubilization and purification of MSA and insulin binding proteins from diploid human fibroblasts will be further investigated. Lectin affinity chromatography and polyacrylamide gel electrophoresis will be used in an attempt to separate mitogen and insulin receptor activity that copurify on Sephadex G-200 chromatography. In addition, insulin and MSA receptors will be purified in a similar manner from other human tissues (fat and/or muscle) to determine the relation of this phenomenon to _in vivo_ insulin receptor action.

Publications:

1. Nagulesparan, M., Savage, P.J., Mott, D.M., Johnson, G.C., Unger, R.H., and Bennett, P.H.: Increased insulin resistance in obese, glucose and intolerant Southwestern American Indians: Evidence for a defect not explained by obesity. J. Clin. Endocrinol. Metab. 51: 739-743, 1980.

2. Hara, H., Hidaka, H., Kosmakos, F.C., Vasquez, B., Mott, D.M., Howard, B.V., and Bennett, P.H.: Characterization of human insulin receptor solubilized from cultured fibroblast and erythrocyte cell membrane preparations. J. Clin. Endocrinol. Metab. 52: 17-22, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54021-04 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Hemoglobin A1 Levels in Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	S.L. Aronoff	Chief	PCRS, NIAMDD
OTHER:	W.C. Knowler	Chief	SFSS, NIAMDD
	B. Vasquez	NIH Postdoctoral Fellow	PCRS, NIAMDD
	D. Mott	Research Biochemist	PCRS, NIAMDD
	B.V. Howard	Assistant Chief	PCRS, NIAMDD

COOPERATING UNITS (if any)

N. Rushforth, Case Western Reserve University, Cleveland, Ohio

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study was undertaken to determine whether the Bio-Rad HbA_1 test could be used to replace the method previously used to determine glycosylated hemoglobin concentration (modifications of Gabbay and of Trivelli). Blood samples were taken from 22 normal male, 24 normal female, 11 diabetic male, and 22 diabetic female Pima Indians who were participating in epidemiological studies of diabetes on the Gila River Indian Reservation in Arizona. Both methods showed increased HbA_1 concentrations in the diabetic groups when compared to the normals, but the kit values were lower, in general, than the values obtained with the previously used method. One set of control samples used revealed a need to run all assays at a constant temperature and to establish a series of frozen controls prior to beginning long-term epidemiological studies.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Studies during the preceding year showed a high correlation between HbA_1 values and fasting and 2 hour post glucose load plasma glucose concentrations. This allowed discrimination between diabetic and nondiabetic groups within the study population. It is planned to use the HbA_1 levels in longitudinal studies as a tool to study the development of diabetes and its complications and as a possible alternative to OGTTs for use in home visits.

In order to determine the most efficient and effective method of glycosylated hemoglobin measurement for the longitudinal epidemiological studies, a comparison was made between the method previously used (modifications of the methods of Gabbay and of Trivelli) and the Hemoglobin A_1 Column Test kit available from Bio-Rad Laboratories. This kit method offers advantages over the previously used method in its safety, ease and speed of determination.

Methods Employed: Glycosylated hemoglobin was measured by two methods. One, a modification of the methods of Gabbay and of Trivelli, involved washing the red cells with physiologic saline,hemolyzing the cells with water and toluene, dialyzing the hemolysate against a cyanide-phosphate buffer, and chromatographing the hemolysate on a Bio-Rex 70 column. The glycosylated hemoglobin was eluted with a phosphate buffer (pH 6.4). The optical density of the two fractions was measured at 415 nm, and the percentage of the total hemoglobin that was glycosylated was calculated.

The second method was the Hemoglobin A_1 by Column test from Bio-Rad Laboratories. A sample of whole blood was added to a hemolysis reagent. Then an aliquot of the hemolyzed sample was placed on a prepacked column followed by an Elution/Developing reagent. Another aliquot was added directly to the Elution/Developing reagent but not to the column. The optical density of the column fraction and that of the sample added directly to the Elution/ Developing reagent was measured at 415 nm, and the percentage of glycosylated hemoglobin was calculated.

All assays were run at room temperature which was recorded. Both methods were used on samples from each patient involved in the study, and the values were then compared.

Major Findings: The study group fro comparison of the two methods consisted of 22 normal males with a mean age of 37.0±12.8 (SD) years, 24 normal females with a mean age of 49.2±11.4 years, 11 diabetic males with a mean age of 51.9±16.3 years, and 22 diabetic females with a mean age of 49.2±11.4 years. All of the subjects were Pima Indians.

Glycosylated hemoglobin levels in the normal subjects using the kit method were 7.61±0.83% in the males and 7.36±1.52% in the females. These values were lower than the values achieved using the modifications of Gabbay and of Trivelli. In that method the mean level in the normal male subjects was 8.77±1.49% and in the normal females 9.11±1.76%. The values obtained using the kit were on average 22% lower in the males and 24% lower in the females than the values using the modified method.

Values by both methods in the diabetic subjects were higher than in the normals with 10.46±1.48% in the diabetic male subjects by the kit method and 12.65±3.71% using the modified method. Similarly, the HbA_1 concentration in the diabetic females was 11.73±3.01% by the kit and 12.90±2.60% with the previously used method.. Again the levels obtained using the kit were on average 24% lower in the diabetic males and 9% lower in the diabetic females than the values obtained with the modified method.

Analyses by the kit method seemed to be quite sensitive to room termperature fluctuations. One control series had manufacturer's values of 7.6±0.5% at 22°C and 8.5±0.5% at 25°C. After 25 assays the mean glycosylated hemoglobin concentration in our hands was 9.97±0.31% at a mean temperature of 26.02±0.59°C. Since the HbA_1 readings using the kit method seemed to increase dramatically with increases in room temperature, a refrigerated incubator has been purchased prior to beginning the long-term epidemiological sampling in order to run all assays at the same temperature.

Significance to Biomedical Research and the Program of the Institute: Both methods of HbA_1 determination, the Bio-Rad kit method and the modifications of Gabbay and of Trivelli, allow clear distinction between the normal and diabetic groups of Pima Indians. Although the kit method seemed to give lower values than the modification method, it is believed to be a safer, easier, and faster method of HbA_1 determination than the modification method and allows discrimination between normal and hyperglycemic subjects. The results for either assay would be improved by running the assays at a constant temperature.

Proposed Course: This project will be continued using the kit method at constant temperature conditions to determine the HbA1 levels in longitudinal studies of patients in order to assess the development of diabetes and its complications. In addition the relationship between HbA_1 measurements and oral glucose tolerance tests will be verified so that the HbA_1 levels can be used as an alternative to the oral glucose tolerance test in home visits. A series of frozen controls will also be established to ensure long-term reliability of the assays.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54023-04 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Very Low Density Lipoprotein Metabolism in Pima Indians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.V. Howard	Assistant Chief	PCRS, NIAMDD
OTHER:	J.S. Reitman	Staff Physician	PCRS, NIAMDD
	S.L. Aronoff	Chief	PCRS, NIAMDD
	L. Zech	Staff Physician	IRMD, NHLBI

COOPERATING UNITS (if any)
Scott M. Grundy, Dept. of Medicine, School of Medicine, University of California San Diego, CA; Marja Riita Taskinen, Dept. of Medicine, University of Helsinki

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 2.1	PROFESSIONAL: 0.6	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pima Indians have a high prevalence of obesity, diabetes mellitus and hyperinsulinemia, but they have low plasma cholesterol levels, reduced LDL synthesis, and decreased incidence of cardiovascular disease. VLDL metabolism in Pima Indians will be investigated in order to further investigate lipoprotein metabolism in this population. Synthetic and catabolic rates for VLDL will be compared in groups having different insulin levels using tritiated glycerol as a precursor. VLDL metabolism will be studied in a group of diabetics to determine the influence of hyperglycemia on VLDL metabolism in this population. Conversion of VLDL to LDL will be studied in nondiabetics and diabetics using ^{125}I VLDL and ^{131}I LDL simultaneously. Postheparin, muscle, and adipose tissue lipoprotein lipase levels will be measured. The results of this study should yield data on VLDL metabolism and its conversion to LDL in this population and explore the relation between these lipoproteins, diabetes and cardiovascular disease.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

1. To study the metabolism of VLDL-TG using a pulse-injection of ^{3}H glycerol in a group of Pima nondiabetics and groups of weight matched Caucasian controls to determine synthetic and a catabolic rates of VLDL-TG in the Pimas and to determine the effects of body weight and plasma insulin levels on these metabolic parameters.

2. To compare VLDL-TG metabolism in a group of untreated male diabetics matched in age and weight to group #1 in order to assess the mechanism of hyperglycemia-induced elevations of VLDL.

3. To compare VLDL-TG metabolism in a group of nondiabetics before and after exercise training to determine whether exercise-induced declines in VLDL are caused by decreased synthesis or increased catabolism.

4. To measure post-heparin lipolytic activity and adipose and muscle lipoprotein lipase activities in a number of Pima Indians over a range of obesity to determine if VLDL-TG metabolism in the Pimas is related to the levels of this enzyme and to evaluate the effects of weight loss on lipoprotein lipase activity.

Major Findings:

1. ^{3}H Glycerol Studies. VLDL metabolism was studied, using ^{3}H-glycerol as an endogenous precursor of triglyceride synthesis, in 15 obese Pima nondiabetic males and compared to that of 20 obese and 13 normal weight, normal lipidemic, nondiabetic Caucasian males. The resultant kinetic data were analyzed using a multicompartmental model which includes two pathways for VLDL-TG synthesis and a process of stepwise delipidation for VLDL catabolism. As compared to obese Caucasians the obese Pimas had a lower rate for VLDL-TG synthesis, and a lower proportion of slow pathway for synthesis. The fractional catabolic rate in the Pimas was higher than in either Caucasian group. When the relation between VLDL-TG metabolism and plasma insulin was examined, plasma insulin levels in the Pima were not correlated with VLDL-TG synthetic rates, catabolic rates or plasma pools. On the other hand VLDL-TG synthetic rates were correlated with plasma free fatty acid levels. Thus, in the Pima population, VLDL-TG synthesis is low, VLDL-TG catabolism is accelerated, and VLDL pools appear to be insensitive to the influence of body weight and hyperinsulinemia.

The kinetic data on VLDL-TG observed from the Pima revealed increased fraction delipidized, a short VLDL residence time and a low proportion converted to IDL; the relation between these observations and VLDL particle size must be explored. Finally, there was a low proportion of slow/fast pathway for VLDL-TG production in all the Pimas studied, and this parameter was elevated in the more obese subjects of both Pima and control populations. Interpretation of this awaits further elucidation of the nature of the two pathways of VLDL-TG production.

To date 11 studies have been completed on untreated diabetics and have been accompanied by measurements of glucose tolerance, and insulin and C-peptide levels. Preliminary analysis of the data suggest hyperglycemia may be accompanied by changes in both synthesis and catabolism of VLDL.

To date 5 patients have completed a study of VLDL-TG metabolism before and after exercise training. Individuals were exercised for a period of 5 to 10 weeks at 60-80% of maximal aerobic capacity. During this period, body weight was maintained constant, but plasma TG levels were observed to fall in all 5. Data analysis awaits results of the multicompartmental modeling of the kinetic data.

The IDL fraction (density 1.006-1.019) has been isolated from 8 of the non-diabetic patients, and the specific activity data from this fraction is being used for the development of a multicompartmental model for IDL metabolism.

2. Lipoprotein Lipase Studies. For our study of the relationship of obesity to fat and muscle cell metabolism, 25 Caucasian and 22 Amerindian males of variable percentage desirable weight each underwent oral glucose tolerance test, fat and muscle biopsies, and I.V. heparin injection for determinations of: fat cell size, number and lipoprotein lipase (LPL) activity; muscle LPL activity; post heparin lipolytic (PHLA) and hepatic lipase (HL) activities. Six of these 22 men plus an additional two Pima males each underwent a period of weight reduction with weight stabilization for two to eight weeks post-weight reduction.

Adipose tissue LPL activity in the Amerindians was closely related to percentage desirable weight when normalized for cell number per gram wet weight (r=0.76, p=.001). The Caucasians demonstrated a similar relationship between adipose LPL and percentage desirable weight (PDW), however LPL activity in the Caucasians was much higher than in the Amerindians at comparable PDW. There was no relationship in the Amerindians between PDW and muscle LPL, peripheral heparin-releasable LPL (HRLA) or hepatic lipase (HL) activities.

HRLA was not related to adipose LPL activity but was related to muscle LPL activity, suggesting that adipose LPL may not be completely released by intravenous heparin by the time of sampling (15 min).

Weight reduction in six Pima males caused adipose LPL activity per cell to decrease in parallel with the decrease in PDW. Muscle LPL activity showed a tendency to increase and hepatic lipase, a tendency to decrease with weight reduction, however, HRLA showed no consistent behavior.

Overall, these results suggest that adipose tissue LPL activity in Southwest Amerindians follows changes in weight and is probably not a primary factor in the etiology of obesity in this group. Furthermore, the rapid catabolic rate for VLDL-TG in this population did not seem to be reflected in elevated LPL.

Significance to Biomedical Research and the Program of the Institute: The results of these studies are relevant to the understanding of in vivo lipoprotein metabolism, and its relationship to diabetes and obesity. The comparison of VLDL metabolism in groups of diabetics and obese Indians will indicate whether or not these lead to increased synthesis or catabolism of VLDL. The studies on the interconversions of VLDL to LDL will determine if LDL synthesis is low in the Pimas and will yield information on the relationship between lipoprotein turnover and cardiovascular diseases.

The findings of elevated VLDL-FCR in obese, nondiabetic Pima Indians raise the question of whether the key lipogenic enzyme LPL is instrumental in mediating the observed increased FCR. In addition, higher adipose LPL levels may be important in the etiology of the obesity observed in the Pima population. The measurement of LPL before and after weight loss will yield information on the role of this enzyme in obesity.

It is known that in normal subjects muscle LPL levels increase with physical training; if adipose tissue LPL levels increase less or even decrease, then exercise training provides a mechanism whereby muscle lipogenesis is increased at the expense of adipose tissue lipogenesis. The measurements for VLDL metabolism and lipoprotein lipase before and after exercise will test this hypothesis.

Proposed Course:

1. Studies of VLDL-TG metabolism: Data analyses will be completed on VLDL-TG metabolism in the group of male diabetics. Data will be compared to the previous age, weight matched group of nondiabetics, and the effect of hyperglycemia and insulin depletion on VLDL catabolism and synthetic parameters will be assessed to elucidate the mechanism of diabetes-induced changes in TG.

Data will be analyzed from the studies of VLDL metabolism before and after exercise training. The kinetic data will be related to the before and after measurements of muscle and fat LPL and plasma lipoprotein levels which were also made on these subjects.

Isolation of IDL fractions will continue dna specific activity data used for further development of an IDL model and analyses of IDL metabolism in this population.

2. Studies of apo B Metabolism in Diabetic and Nondiabetic Pimas: Having acquired data on VLDL to metabolism and its relationship to diabetes and obesity, the next step in the understanding of lipoprotein metabolism and the mechanism of diabetes and obesity-induced changes is the investigation of apo B metabolism. This will be accomplished through double-label studies using ^{125}I VLDL and ^{131}I VLDL in groups of nondiabetic and diabetic male Pimas. These will be accompanied by analyses of the size of VLDL and LDL apo B in this population using gel filtration and electrophoretic techniques. These studies will allow quantification of the rates of VLDL and LDL apo B synthesis and catabolism in Pimas and the determination of how these are altered in obesity or diabetes. The extent of conversion of VLDL to LDL will be measured and this may contribute to the understanding of the regulation of cholesterol levels in this population. Examination of molecular forms of apo B will determine how these are related to VLDL and LDL metabolism and to nutritional or diabetic status.

3. Studies of Free Fatty and Metabolism: Plasma free fatty acids, which are the immediate precursors of the fatty acids in VLDL triglyceride, will be quantified and turnover will be assessed by a pulse injection of albumin bound ^{14}C free fatty acid. Free fatty acid levels and turnover rates will be compared in thin and obese groups to determine if adipose tissue free fatty acid production reflects or correlates with other indices of insulin resistance.

Publications:

1. Howard, B.V., Zech, L., Davis, M., Bennion, L.J., Savage, P.J., Nagulesparan, M., Bilheimer, D., Bennett, P.H., and Grundy, S.M.: Studies of very low density lipoprotein triglyceride metabolism in a population with low plasma lipids - lacl of influence of body weight or plasma insulin. J. Lipid Res. 21: 1032-1040, 1980.

2. Howard, B.V., Zech, L., Davis, M., and Grundy, S.M.: Very low density lipoprotein metabolism in the Pima Indians. In Proceedings of the Workshop on Lipoprotein Kinetics. Eds. M. Berman, S. Grundy, and B.V. Howard. Academic Press, NY, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54024-03 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

In Vitro Insulin Resistance in Pima Indians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.L. Aronoff	Chief	PCRS, NIAMDD
OTHER:	F.C. Kosmakos	Guest Worker	PCRS, NIAMDD
	J. Foley	Guest Worker	PCRS, NIAMDD
	J.S. Reitman	Staff Physician	PCRS, NIAMDD
	D.M. Mott	Research Biochemist	PCRS, NIAMDD
	B.V. Howard	Assistant Chief	PCRS, NIAMDD
	S. Cushman	Associate Chief	CMOS, NIAMDD
	L. Salans	Associate Director	DEM, NIAMDD

COOPERATING UNITS (if any)

Joseph Larner, Univ. of Virginia Medical School, Charlottesville, VA

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	0.7	2.5

CHECK APPROPRIATE BOX(ES)

XX☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obesity in the Pima Indian is associated with a massive increase in adipose tissue mass (25-50% of total weight). The increased fat mass is due to both an increase in adipose cell size and cell number. Adipose lipoprotein lipase activity increases with obesity and is correlated with adipose cell size. Methods of studying various parameters of carbohydrate and lipid metabolism have been established using isolated human adipocytes and membrane vesicles and will be used to study human obesity.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives:

1. Cellular Characterization of Obesity in the Pima Indians. An increase in fat mass associated with either an increase in adipose cell number and/or adipose cell size. The role of cell number and size in the development of obesity in the Pimas will be assessed by measuring total body fat and total adipose cell number and size. Anthropometric measurements will be made simultaneously to determine its quantitative value in assessing obesity.

2. Examination of the Role of Adipose Lipoprotein Lipase in Obesity of the Pima Indian. Lipoprotein lipase is the major enzyme responsible for the assimilation of triglyceride fatty acid into tissue. Enzyme activity will be measured in adipose tissue obtained from 3 sites (gluteal, abdominal, and triceps) and correlated with the degree of obesity.

3. Measurement of Glucose Transport in Isolated Human Adipose Cells. Free fatty acids released from VLDL by lipoprotein lipase are assimilated by adipocytes and esterified with α-glycerol phosphate to form triglycerides. Availability of α-glycerolphosphate is a rate limiting step in fatty acid esterification and is determined by the rate of insulin sensitive glucose transport. The ability of adipocytes from Pima Indians at different stages of obesity to transport glucose under a variety of metabolic conditions (i.e. insulin, growth hormone, cortisol and glucose concentrations) will be tested in order to determine if this rate limiting step in energy storage is affected by obesity.

4. Measurement of Lypolysis in Isolated Human Adipose Cells. Free fatty acids released from adipose cells are an important source of energy during fasting conditions. Alterations in the ability of adipose cells to respond to fasting will be tested at different stages of obesity under various metabolic conditions since inappropriate responses may lead to an increased deposition of fat.

5. Measurement of the Intracellular Distribution of Glucose in Isolated Human Adipose Tissue. Glucose distribution to various metabolic pathways as a function of obesity will be measured to determine if there are non-transport related changes in insulin stimulation of lipid anabolic pathways.

6. Characterization of the Glucose Transport System in Adipocytes. The mechanism by which glucose is transported across the membrane will be investigated using membrane vesicles from rat and human adipocytes. The membranes will be used for studies of cytochalasin B binding and kinetics of glucose transport to elucidate the mechanism of transport and subsequently, the factors that regulate it. Such studies will hopefully allow more meaningful measurements of changes in the transport system with the onset of obesity.

7. Characterization of the "Basal Transport Factor". The rate of glucose transport in adipocytes is controlled by regulating the distribution of glucose transport systems between the plasma membrane (pm) and the endoplasmic reticulum (ER). Studies are planned to examine the role of insulin and insulin antagonists on the distribution of glucose transport systems and on the development of insulin resistance.

Methods:

1. Adipose cell number and cell size will be measured on osmic acid fixed cells using a Coulter Counter coupled to a cell sizer.

2. Total body fat will be measured indirectly by estimating lean body mass with deuterium oxide.

3. Adipose lipoprotein lipase will be assessed by measuring the release of labelled fatty acids from ^{14}C-triolein.

4. Glucose transport in isolated adipocytes will be measured with ^{14}C-3-O-methylglucose.

5. Lypolysis will be measured as glycerol released into the medium during incubation with various insulin and catacholamine concentrations.

6. Glucose metabolism will be assessed by measuring ^{14}C-glucose conversion to CO_2 glycerol, fatty acids, nucleotides and lactate.

7. Glucose transport sites will be measured with labelled cytochalasin B.

Major Findings:

1. Obesity in the Pima Indians. Total body adiposity has been assessed in Pimas over a range of obesity. The degree of adiposity ranges between 25-50% of the total body weight. In general, for any given body weight, Pimas have a greater degree of adiposity than weight-matched Caucasians. Analyses of the number of whole body adipocytes and adipocyte cell size suggest that the increase in fat mass is due to an increase in both cell size and cell number. Whereas cell number increases with body weight, the total number of fat cells (20-40 billion per subject) falls within the range reported for hypertrophic obesity.

2. Lipoprotein Lipase Activity Associated with Pima Obesity. We have found an increase in lipoprotein lipase activity of abdominal adipose tissue with obesity. Similar results were observed with gluteal and triceps adipose tissue. Studies are in progress to correlate the results of adipose cell size, cell number, and lipoprotein lipase activity of gluteal, abdominal, and triceps fat with total body adiposity and anthropometric measurements.

3. Glucose Transport Studies with Human Adipose Tissue: Glucose transport rates can be measured in very obese Pima Indians. The transport characterics are similar to those reported by others for Caucasian fat. Insulin responses are of the same magnitude as those found in our glucose metabolism studies when very low glucose concentrations are used. Dose response measurements show insulin sensitivity in the 50-150 pM range for Pima Indians. Plasma membrane vesicles have been made from both rat and human adipose tissue. The transport characteristics of the rat membrane vesicles are consistent with those found in whole cells. Cytochalasin B binding studies suggest that human adipose vesicles have a similar transport mechanism as found in rat adipose tissue.

Significance to Biomedical Research and Program of the Institute: Obesity and/or diabetes have profound physiological and psychological ramifications in a large segment of the U.S. population. The Pima Indians represent a stable, homogeneous group with which to elucidate the pathogenesis of these altered metabolic states. The aim of these studies is to first characterize these states. Once characterized, longitudinal studies will be initiated to examine the development of the changes in metabolism, and potentially, the factors which produce these changes.

Proposed Course: The cross-sectional studies of total body adiposity, adipose cell size and adipose lipoprotein lipase activity will be extended to longitudinal studies of obesity in the Pima population. These measurements will be made in conjunction with cross-sectional and longitudinal studies of insulin sensitive parameters of carbohydrate and lipid metabolism in adipocytes derived from diabetic and nondiabetic Pima Indians. Since the insulin receptor does not appear to contribute to insulin resistance it is anticipated that studies involving fat cells from Pima Indians will help to elucidate the alterations in metabolic pathways associated with obesity and conditions of insulin insensitivity.

Publications: None

This study is supported entirely by NIH N01-AM-6-2219.

Starting Date: September 1, 1980
Expiration Date: October 31, 1981

Contractor: University of Texas Health Science Center
Southwestern Medical School

Money Allocated: $2,794,619 Man-years Purchased: 32.2

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54025-03 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

$(Na^+ + K^+)$ ATPase Activity in Obese Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David M. Mott	Research Biochemist	PCRS, NIAMDD
OTHER:	Barbara V. Howard	Assistant Chief	PCRS, NIAMDD
	I. Klimes	Guest Worker	PCRS, NIAMDD
	R. Clark	Postdoctoral Fellow	PCRS, NIAMDD
	M. Nagulesparan	Guest Worker	PCRS, NIAMDD

COOPERATING UNITS (if any)

Human Diabetes Study Center, University of Texas Health Science Center

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	.8	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The level of Na-pump activity has been implicated in mechanisms which could contribute to human obesity. $(Na^+ + K^+)$ ATPase activity is reduced in intact red cells and isolated erythrocyte membranes from obese Pima Indian subjects. Fibroblasts, white cells and adipocytes are being examined to determine if there is a widespread tissue loss of sodium pump activity. These cells are also being tested for a response of the Na-pump to hormones which stimulate thermogenesis: Studies of the sodium pump activity in red cells from thin and obese families and in diploid fibroblasts will be used to determine possible genetic differences in the sodium pump which relate to obesity.

Project Description:

Objectives: Previous studies indicated that the Na-pump may make a major contribution to basal and hormone stimulated cellular thermogenesis. The recent demonstration that the genetically obese (ob/ob) mouse has reduced levels of Na^+, K^+-ATPase in muscle and liver suggests that a defect in a single enzyme could reduce cellular thermogenesis. A resulting shift in the balance between energy intake and thermogenesis might determine whether an animal will gain or lose body fat. If the activity level of the Na-pump has a causal relationship with human obesity, obese subjects with normal thyroid function should have decreased plasma membrane ($Na^+ + K^+$)ATPase activities. Alternatively, failure of the Na-pump to respond to hormones which regulate thermogenesis might be considered as mechanisms which could contribute to human obesity.

Pima Indians are a genetically homogeneous group characterized by a high frequency of obesity and hyperinsulinemia. They offer a unique opportunity for testing hypotheses relating genetic alterations to human obesity. We have established that obese Pima Indian subjects have reduced basal red cell Na-pump activity. The basal ATPase activity of diploid fibroblasts, while cells and adipocytes from obese subjects will be examined and compared to red cells from the same subject. In addition, these cells will be characterized *in vitro*, and where possible *in vivo*, for possible alterations in response to hormones which control thermogenesis. Results of this study will be used to assess the extent that abnormal regulation of the Na-pump is responsible for obesity in Pima Indians.

Methods Employed and Results:

1. Reduced Na^+, K^+-ATPase activity in Intact Red Cells and Isolated Membranes from Obese Man. Na^+, K^+-ATPase activity was measured in red blood cells from 20 nondiabetic euthyroid male Pima Indians with varying degrees of obesity, their body mass indices (BMI) ranging from 20 to 60 kg/m^2. The Na^+, K^+-ATPase, measured both by ^{86}Rb uptake in intact cells and APR hydrolysis by purified membranes, was inversely correlated with BMI ($r=-0.62$; $p<0.005$ and $r=-0.75$; $p<0.0001$) respectively. The activity of the Na-pump from isolated red cell membranes was significantly correlated with the rate of ^{86}Rb uptake by erythrocytes ($r=0.55$, $p<0.05$). These results confirm that obesity is associated with decreased Na^+, K^+-ATPase in intact red blood cells, and provide the first demonstration of reduced sodium pump activity in isolated red cell membrane preparations from obese man.

2. Plasma Factor Alters Mg^{++} Dependence of Red Cell Na-pump. The Mg^{++} dependence of Na^+, K^+-ATPase activity in laked red cell preparations (contain hemolyzed red cells and plasma) was measured as the ouabain inhibited release of inorganic phosphate. The 11 normal weight subjects had a sharp peak at about 0.9 mM mg^{++} with marked inhibition at 1.2 mM Mg^{++}. A similar Mg^{++} dependence was observed for 8 obese subjects except that their maximum activity was shifted to about 0.8 mM Mg^{++}. Similar studies have been conducted using isolated red cell membranes from normal and obese subjects. In contrast to the laked cell preparations, the maximum Na^+, K^+-ATPase activity in isolated membranes from both normal and obese subjects is reached at 0.9 mM Mg. Compared

to the normal subjects, however the activity for the obese subjects is reduced at all Mg^{++} concentrations tested from 0.6 to 4.6 mM. In addition, the Mg^{++} inhibition of laked cell preparations is not apparent in the isolated membrane preparations. These results suggested that the concentration of Mg^{++} required for maximum activation and inhibition of Na^+, K^+-ATPase was altered by factors removed during membrane purification. This hypothesis was supported by experiments using laked cells prepared in plasma (previous method) or following removal of the plasma by several washes. The laked red cells which were hemolyzed after plasma was removed were not inhibited by elevated Mg^{++} concentrations.

3. Na-pump Activity in Red Cells During the Thermic Effect of a Meal: Sodium pump activity was measured in red blood cells from 8 nondiabetic subjects (7 males and 1 female) with varying degrees of obesity (BMI ranging from 23 to 67 kg/m^2) after an overnight fast or 90 minutes after ingestion of a standard meal. Fasting values for Na^+, K^+-ATPase measured in isolated membranes or intact red cells reproduced the previously observed correlation with BMI. However, no consistent stimulation of the sodium pump activity in the postprandial state was observed. These results suggest that the Na-pump does not contribute to the thermic effect of a meal or that the red cell is not an appropriate system for answering this question.

4. Na^+-pump Activity in Diploid Fibroblasts: Methods have been established for measuring ^{86}Rb uptake by fibroblast monolayer cultures. Rates of ouabain sensitivity ^{86}Rb uptake are linear over a 45 minutes time period and range from 10 to 40 pg Rb/min/10^5 cells. Maximal stimulation of the Na-pump is achieved with a 15 minute incubation in 10^3ng/ml insulin resulting in rates of ^{86}Rb uptake which are 30 to 85% above baseline. Half maximal stimulation requires 0.5 to 3.0 ng/ml insulin.

Significance to Biomedical Research and the Program of the Institute: The demonstration of reduced red cell (Na^+, K^+)ATPase activity in intact cells and isolated membranes from obese Pima Indians suggests that a genetic lesion may effect enzymatic hydrolysis of ATP and could contribute to the excessive obesity in the Pimas. Studies of other tissues will clarify the importance of this abnormality in obesity in the Pima population.

Proposed Studies:

1. Family Study: Several obese and thin families will be studied to determine if the pre-obese child (i.e. the child of obese parents) has Na-pump activity which is comparable to the obese adult or to the thin child from the thin family. Results from these studies will determine if the reduced Na-pump activity of obese subjects is the result of the obese state or a possible cause.

2. White Cell Na-pump Activity: White cells are a readily available source of nucleated cells which may be responsible to hormonal regulation of the Na-pump. These cells will be isolated on Ficol-Hypaque gradients and used to relate basal Na-pump activity (^{86}Rb-uptake) to obesity. In addition, they will be used to measure activity changes in vitro in response to insulin, T^3 and catecholamines Results on the time course, sensitivity and magnitude of stimulation of the

Na-pump in white cells *in vitro* will be used to design experiments which will relate the thermic effects of these hormones to changes in white cell-Na-pump activity *in vivo*. Further details of studies on the thermic effects of these hormones are described in section

3. Adipocyte Na-pump Activity: Isolated human adipocytes will be used to determine whether (Na^+, K^+)ATPase activity, as determined by ouabain inhibitable ^{86}Rb-uptake, correlates with relative obesity of the donor. In addition stimulation of Rb-uptake by insulin will be measured. It is possible that insulin resistance, known to be present in obesity, may be reflected in adipocyte ^{86}Rb-uptake. If this is the case, it would support the hypothesis that reduced sensitivity to insulin caused reduced (Na^+, K^+)ATPase activity in insulin sensitive tissues which in turn contributes to the development of obesity.

4. Diploid Fibroblast Pump Activity: The techniques which have been established for measuring basal and insulin stimulation rates of rubidium uptake by cultured cells will be used to compare the Na-pump activity in ten diploid fibroblast lines from thin and obese donors. A comparison of basal, half maximum and maximum insulin stimulated rates of rubidium uptake by cells thin and obese donors will be used to determine possible genetic differences in Na-pump activity which relate to obesity. In addition, these cells will be characterized in terms of T^3 and catecholamine stimulation of the Na-pump.

Publication: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54026-02 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chlorpropamide-alcohol Flushing - A Predictor for Non-insulin Dependent Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.L. Aronoff	Chief	PCRS, NIAMDD
OTHER:	M. Nagulesparan	Guest Worker	PCRS, NIAMDD

COOPERATING UNITS (if any)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.1	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Some diabetics taking chlorpropamide have an intense facial flush after alcohol. This chlorpropamide induced alcohol flushing (CPAF) is reported to be a dominantly inherited trait associated with non-insulin dependent diabetes. CPAF has also been reported to be present before the onset of glucose intolerance and therefore recommended as a genetic marker for some cases of non-insulin dependent diabetes.

The Pima Indians, a population with the highest reported prevalence of diabetes in the world have the non-insulin dependent type of diabetes. We therefore used the chlorpropamide-alcohol flushing test to study this population, to determine the relationship between CPAF and non-insulin dependent (Type II) diabetes.

Studies in the Pima have failed to show any relationship between CPAF and the presence of diabetes. Other groups have also not observed this association and therefore have failed to confirm the original observations of Leslie and Pyke.

Project Description:

Objectives: Some diabetics taking chlorpropamide have an intense facial flush after alcohol. This chlorpropamide induced alcohol flushing (CPAF) is reported to be a dominantly inherited trait associated with non-insulin dependent diabetes. It is much less frequent in insulin dependent diabetics and nondiabetics. CPAF has also been detected before the onset of glucose intolerance and therefore may possibly be useful as a genetic marker for some cases of non-insulin dependent diabetes. The nature of the association between CPAF and non-insulin dependent diabetes is not clear but has been suggested to be due to increased sensitivity to endogenous opiates such as enkephalin.

The Pima Indians appeared to be an excellent group to further examine the CPAF test since they are a homogenous population with the highest reported prevalence of non-insulin dependent diabetes in the world. Further, since 50% of adult Pimas have diabetes and a nondiabetic Pima Indian with two diabetic parents (genetic prediabetic) has approximately 5 times the risk of developing diabetes as a nondiabetic Pima with two nondiabetic parents, the genetic prediabetic Pima Indian is an ideal subject to determine the predictive capability for diabetes of this test.

Purpose of Study:

1. To determine the relationship between CPAF and non-insulin dependent diabetes.

2. To determine the usefulness of CPAF as a predictor of non-insulin dependent diabetes by studying the genetic prediabetic Pima Indian.

Methods: Pima Indian subjects with normal glucose tolerance, impaired glucose tolerance and diabetes were studied. On the first day, the subjects were given 40 ml of sherry over a one minute period and the skin temperature at the face and wrist measured for 60 minutes. Then, on the evening before the CPAF test, chlorpropamide (250 mg) was given orally. The following morning the subjects drank 40 mls of sherry over one minute (12 hours following the ingestion of chlorpropamide). Skin temperature was measured at the same location (2 cm below the outer canthus of the eye and 8 cm proximal to the wrist on the flexor aspect of the forearm). An increment of skin temperature of >2°F was considered a "flush".

Results: In our preliminary study of chlorpropamide alcohol flusing in the Pima Indians, our findings did not agree with the results first presented by Leslie and Pyke. Since then several other groups who have also studied the chlorpropamide alcohol flush have not been able to confirm the original observations by Leslie and Pyke. It is now apparent that chlorpropamide alcohol flushing will not prove to have much relevance to the study of Type II diabetes. We have therefore stopped all further studies of chlorpropamide alcohol flushing in Pima Indians.

Proposed Course: No further studies of chlorpropamide alcohol flushing will be done in the Pima Indians.

Publications:

The findings of this study were presented at a workshop on "Chlorpropamide-Alcohol Flushing and Diabetes" sponsored by the National Diabetes Data Group of the N.I.H. and the International Diabetes Epidemiology Group, June 13-14, 1980 in Washington, D.C.

NIH Contract No.: N01-AM-6-2219

Starting Date: September 1, 1980
Expiration Date: October 31, 1981

Contractor: University of Texas Health Science Center
Southwestern Medical School

Money Allocated: $2,794,619

Man-years Purchased: 32.2

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54027-02 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of HGH Therapy on Somatostatin and Pancreatic PP and Lipoprotein Levels in GH Deficient Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	S.L. Aronoff	Chief	PCRS, NIAMDD
OTHER:	B.V. Howard	Assistant Chief	PCRS, NIAMDD
	M. Nagulesparan	Guest Worker	PCRS, NIAMDD

COOPERATING UNITS (if any) R. Gingerich, Dept. of Pediatrics, Washington Univ. School of Medicine, St. Louis, MO; R.H. Unger, Human Diabetes Study Center, Univ. of Texas, Southwestern Medical School, Dallas, TX

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.2	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Growth hormone deficient subjects who meet the criteria for growth hormone therapy from the National Pituitary Agency are being selected for study. Serum lipoprotein composition is being measured before, during and after growth hormone therapy. Similarly, serum somatostatin and pancreatic polypeptide levels following a test meal are being measured in these same subjects to determine the effects of growth hormone therapy on these parameters.

PHS-6040
(Rev. 2-81)

Project Description:

Previous clinical studies have indicated that growth hormone has many metabolic effects besides the stimulation of growth. Effects of growth hormone on carbohydrate, protein, and lipid metabolism have been demonstrated. Interactions with several hormones have also been noted.

Marked hyperlipidemia has been clearly demonstrated in growth hormone deficient adult dwarfs. Despite this hyperlipidemia and the abnormalities of carbohydrate metabolism found in adult dwarfs, the prevalence of arterio-abnormalities in carbohydrate and lipid metabolism. A recent study examined the effect of growth hormone therapy on serum triglycerides and cholesterol levels in growth hormone deficient children and found only minor changes in the levels of these lipids after treatment. Lipoprotein fractions were not separated in this study. Measurement of lipoprotein fractions in growth hormone deficient subjects before and during growth hormone therapy would be of interest because growth hormone deficient individuals might have altered lipoprotein distributions which might possibly protect growth hormone deficient dwarfs form vascular disease; morover, alterations in their lipoprotein fractions may occur with growth hormone therapy.

Interrelationships between growth hormone and other hormones such as insulin have been well recognized. Somatostatin is known to suppress growth hormone secretion and a recent study in hypophysectomized dogs reported finding suppressed serum somatostatin levels suggesting a possible feedback relationship between these two hormones. Recent developments in the radioimmunoassay for somatostatin using ^{125}I-Tyrosine-11-somatostatin tracer to avoid problems of aminopeptidase degradation now make possible the determination of human serum somatostatin levels and therefore provide a methodology to determine if growth hormone deficiency or treatment are associated with altered somatostatin levels.

An association between growth hormone and pancreatic polypeptide has been suggested. One study has found pancreatic polypeptide secretion elevated in growth hormone deficient children. Furthermore, they found that growth hormone treatment did not suppress pancreatic polypeptide to normal levels in response to insulin hypoglycemia. Further examination of pancreatic polypeptide levels during growth hormone therapy might help to elucidate the relationship of these two hormones.

Objectives:

1. To examine the composition of serum lipoproteins before and during growth hormone therapy in growth hormone deficient subjects.

2. To examine the relationships between growth hormone and somatostatin and growth hormone and pancreatic polypeptide by measuring these hormones before, during and after growth hormone therapy in growth deficient subjects.

Methods Employed: Growth hormone deficient euthyroid subjects whose height is more than two standard deviations below the mean for age, have a current growth rate of <4 cm/yr, have a bone age delay of two or more years and an inadequate growth hormone response to two definite stimuli (insulin hypoglycemia, arginine infusion or L-Dopa) are considered for study. Each subject is admitted and placed on a weight-maintaining diet consisting of 45% CHO, 40% Fat and 15% Protein for three days. Then, after an overnight fast blood samples are obtained for plasma lipids and lipoprotein determinations and the measurement of somatostatin, pancreatic polypeptide, insulin, glucagon and glucose. The patient is then fed a test meal of 12-15 gm/kg composed of 25% Protein, 40% Fat and 35% CHO with blood sampling every 30 minutes for three hours for measurement of somatostatin, pancreatic polypeptide, insulin, glucagon, and glucose concentrations.

Major Findings: This study has commenced only recently. To date only one growth hormone deficient subject has been recruited for this study. Therefore, no data is presently available.

Significance to Biomedical Research and the Program of the Institute: This study should provide insight into some of the hormonal changes occurring in the growth hormone deficient state. Furthermore, it should provide better understanding of the interaction of growth hormone with somatostatin and pancreatic polypeptide. The study should also help elucidate the influence of growth hormone on plasma lipoprotein composition in humans.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54028-02 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Determination of Gastric Acid Secretion in Pima Indians

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.L. Aronoff	Chief	PCRS, NIAMDD
OTHER:	M. Nagulesparan	Guest Worker	PCRS, NIAMDD
	H. Sasaki	Guest Worker	PCRS, NIAMDD

COOPERATING UNITS (if any) R.H. Unger, Human Diabetes Study Center, Phoenix, AZ; Eugene Straus, V.A. Hospital, Bronx, NY; Roslyn Yalow, V.A. Hospital, Bronx, NY; I.L. MacGregor, V.A. Med. Center, Tucson, AZ; A. Dubois, Uniform Services Hospital, Bethesda, MD.

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	0.2	3.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The development of obesity in the Pima Indians may in part be determined by the rates of gastric emptying. Furthermore, peptic ulcer disease is known to be low in this population, but increasing. Studies of gastric emptying and gastric acid secretion to date suggest that rates of liquid gastric emptying are not altered in obesity. Diabetes appears to be associated with decreased acid secretion and hypergastrinemia. The low acid production in the Pima Indians may explain the low incidence of gastroduodenal ulceration.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Obesity and carbohydrate intolerance are common among the Pima Indians. It is possible that the development of obesity may in part be determined by the rates of gastric emptying. The rapid emptying of food from the stomach would decrease the time of postprandial satiety consequent upon gastric distension by ingested food. Also, the more rapid transit of nutrients into the small intestine and the probably more rapid absorption would result in a peak of nutrient delivery to the body and encourage energy storage as neutral fat. An uncontrolled observation by radiologists is that barium sulfate suspension leaves the stomach of Pima Indians more rapidly than that observed in the Caucasian population. Furthermore, peptic ulcer disease is low in the Pima Indian population, but increasing. It is also known that postprandial serum gastrins in Pima Indians who are diabetic are high. For the above reasons, we plan to evaluate concurrently gastric empyting and gastric secretion of acid in Pima Indians over a range of obesity with and without diabetes. We expect to obtain answers to the following:

1. Do the fractional emptying rates correlate with the degree of obesity?
2. Are acid output and fractional emptying rates significantly different in the Pima Indians compared to a population with a greater frequency of peptic ulcer?
3. Does diabetes and glucose intolerance correlate with alterations in gastric emptying and gastric secretion?
4. If the increase in the incidence of peptic ulcer disease among the Southwestern American Indian population continues, will a subsequent study of gastric acid secretory rates show corresponding changes in gastric acid secretion?

Methods: A group of thirty-eight subjects (13 Caucasian, 13 Obese Pimas, 12 Diabetic Pimas) were admitted to the metabolic ward to study the hormonal response to a standard meal. Thirty-one other subjects (8 Caucasians and 23 Pima Indians) had studies of intragastric volume, gastric emptying and secretion determined using a dye dilution technique.

The rate of gastric emptying and the rate of secretion of acid and volume were measured simultaneously using a dye dilution technique. A nasogastric tube was passed into the stomach of a fasting subject. Blood samples for serum glucose, serum insulin and serum gastrin were drawn at 15-minute intervals during the test. The test itself consisted of a 40-minute fasting period, a subsequent 60-minute period following intragastric administration of a 250 ml test water load (containing 40 mg phenol red per liter) and a final 60-minute period. At the beginning of the final 60-minute period histalog Betazole 1 mg/kg body wt was given im. At ten minute intervals during the study, gastric contents were aspirated and 5-20 ml of a test solution identical in compositio to the test meal was injected into the stomach. At the end of the procedure, the stomach was emptied completely. The concentration of phenol red and H^+ ion was determined in all samples. From these data, we calculated the intragastric volume and acid content at each ten-minute time point, the amount of emptying in each ten-minute interval, and, by difference, infer the rates of secretion of acid and water.

Results: The plasma gastrin levels before and after the test meal were higher in the Pima Indians (both diabetic and nondiabetic) than in the Caucasians.

The basal acid concentration and acid secretion rate were lower in the diabetic obese Pimas than in nondiabetic obese Pimas (13.11±4.77 µEq/ml and 25.88±9.94 µEq/min vs 19.96±6.18 µEq/ml and 29.58±9.32 µEq.min, N.S.) and both groups were lower than those observed in non-obese Caucasians (30.37±3.72 µEq/ml and 56.81±6.19 µEq/min, $p<0.01$). Following a 250ml water load and Histolog administration (1 mg/kg), gastric acid concentration and acid secretion rate were also lower in both the nondiabetic obese Pimas (N.S.) and diabetic obese Pimas ($p<0.01$) as compared to the non-obese, nondiabetic Caucasians. There was no difference among the three groups in fractional gastric emptying rate during the basal state (9.85±1.1, 11.13±2.2 and 9.69±2.61%/min), but following a water load, the diabetic obese Pimas (8.96±1.2%/min, $p<0.02$) were significantly lower than those in other groups. Neither fractional rate of gastric emptying, nor gastric acid concentration was related to degree of obesity (BMI: Wt Kg/Ht m^2 and body surface area: m^2) in any groups.

These observations suggest that rates of noncaloric liquid gastric emptying are not altered in obesity. Diabetes appears to be associated with decreased acid secretion and hypergastrinemia. The low acid production in the Pima Indians may explain the low incidence of gastroduodenal ulceration.

Proposed Course: The preliminary studies of gastric emptying and secretion were done using a non-nutrient (water). It is now proposed to study the effect of liquids containing nutrient (glucose) on the rate of gastric emptying and secretion in subjects with varying degrees of obesity and glucose tolerance.

This project has been supported entirely by:

NIH Contract No: N01-AM-6-2219 Starting Date: September 1, 1980
Expiration Date: October 31, 1981

Contractor: University of Texas Health Science Center
Southwestern Medical School

Money Allocated: $2,794,619 Man-years purchased: 32.2

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54029-01 DDB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Nutrition-Induced Alterations in Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	S.L. Aronoff	Chief	PCRS, NIAMDD
Other:	B. Daniels	Guest Worker	PCRS, NIAMDD
	M. Nagulesparan	Guest Worker	PCRS, NIAMDD
	J.S. Reitman	Staff Physician	PCRS, NIAMDD
	F.C. Kosmakos	Guest Worker	PCRS, NIAMDD
	B.V. Howard	Assistant Chief	PCRS, NIAMDD
	R.F. Goldman	Director	USA Ergonomics Lab.
	M. Lawson	Chief, Nuclear Med.	Good Samaritan Hosp.

COOPERATING UNITS (if any)
E. Danforth, Jr., E.A.H. Sims, E. Horton, Dept. of Medicine, U. of Vermont, Burlington, VT., L. Landsburg, Dept. of Medicine, Harvard University.

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 4.3	PROFESSIONAL: 0.8	OTHER: 3.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been proposed that the high incidence of obesity in the Pima Indians may be due to genetic selection for a "thrifty gene", a gene that would allow the Pimas to store energy very efficiently; these efficient individuals would presumably be more capable of surviving famine than lean, less-efficient individuals. In order to help determine whether or not such a genetic difference exists, thermogenic responses to various stimuli, including food, norepinephrine, and exercise, are being investigated in obese Pima volunteers. These thermogenic responses are being measured during periods of weight maintenance, of overnutrition, and of undernutrition. Thyroid hormone metabolism and resting metabolic rate is also being studied, since previous studies with Caucasian volunteers have shown that these parameters are affected by dietary manipulation. The results from this research are being compared to those obtained from lean and obese Caucasians involved in studies in Vermont.

Project Description:

Objectives:

1. The overall objective of the project is to explore in the Pima Indians the factors which contribute to their prevalent obesity and the intimately associated Type II diabetes.

2. To study the thermogenetic responses of the Pimas and the alterations in thyroid and catecholamine metabolism induced by short-term stimuli (food, norepinephrine infusions, and exercise) during periods of weight maintenance, over-nutrition and under-nutrition, and to compare these responses with those of Caucasians.

3. To dissociate, where possible, those changes that are a consequence of the altered body mass from those that result from total caloric intake or from change in composition of the diet.

4. To clarify further the possible mechanisms that might be responsible for nutritionally-induced alterations in thyroid and catecholamine metabolism and the mechanism(s) by which these changes influence thermogenesis and the body economy.

Methods:

1. Anthropometric measurements have included height, weight, circumference measurements, skinfold thickenss, and underwater weighing to determine percentage of body fat and fat free mass (FFM), and triple-site fat biopsies to estimate fat cell size and number.

2. Measurements of oxygen consumption and carbon dioxide production are made using indirect calorimetry, specifically a hood system with a pneumotach or a dry gas meter for determining flow rate.

3. During the three-week over-nutrition phase, the subject consumes the calories required for weight maintenance plus an additional 1,000 kcal per day in the form of a balance liquid formula (Ensure Plus). During the under-nutrition phase, the subject takes only thirty percent of his daily weight maintenance calories each day for six weeks.

4. Metabolic rates are measured each morning for two fifteen-minute periods, between 6:30 and 7:30 a.m., and for two more fifteen-minute periods between 7:45 and 8:45 a.m., and as a baseline for the thermogenic stimuli, which include:

   A. food, 800 kcal of Ensure Plus
   B. norepinephrine, doses of 0.05, 0.10, 0.15, and 0.20 μg/kg FFM/min, each infused for 30 minutes
   C. exercise, an efficiency test in which the subject cycles for 5-10 minutes at each of four levels (0, 30, 60, and 90 watts).

5. Blood samples are taken at appropriate times to be analyzed for catecholamines, thyroid hormones, glucose, insulin, free fatty acids, tryglycerides, and ketones. Urine samples are also collected for catecholamine analysis.

Major Findings:

1. Six subjects have completed the weight-maintenance phase thus far, five have completed the over-nutrition phase and two have completed the under-nutrition phase of this study.

2. The following table will be submitted to be considered for presentation at the fall meetings of the American Federation of Clinical Research:

				Thermogenic response (ΔmlO_2/min/kg FFM)				
Subject	Wt.	FFM	Baseline MR	Food	Dose NE (µg/kgFFM/min)			
	(kg)	(kg)	(mlO_2/min/kgFFM)	(800 kcal)	(0.05	0.10	0.15	0.20)
1	158	100	4.33	0.62	0.25	1.05	1.19	--
2	77	59	4.05	0.56	0.17	0.24	0.47	0.80
3	112	78	3.72	0.45	0.47	0.78	--	--
4	146	99	3.89	0.41	0.06	0.05	0.32	0.69
5	121	63	4.70	0.59	0.29	0.59	0.67	0.75
6	93	60	4.78	0.23	0.00	--	0.62	0.40
x	118	77	4.26	0.41	0.21	0.54	0.65	0.66

Metabolic rate (MR) was measured in six obese Pima Indians for 3-1/2 hours following the ingestion of 800 kcal Ensure Plus, or during and for 30 minutes after a 30 minute intravenous infusion of norepinephrine (NE) in doses of 0.05, 0.10, 0.15, 0.20 µg/min/kg fat-free mass (FFM). FFM was measured by underwater weighing and MR (ml O_2/min/kg FFM) by indirect calorimetry. Thermogenic response is expressed as the difference between the mean MR during the test, and the baseline MR, which was measured for 30 minutes prior to the stimulus.

3. It is too early to draw any conclusions from these preliminary results since they are awaiting statistical evaluation and the majority of the blood samples have not been analyzed; it will be particularly important to know the serum concentrations of NE produced by the infusion. However, comparing preliminary results with those of the Vermont group, it appears that the thermogenic response of Pima Indians to norepinephrine will not be significantly different from that of lean Caucasians, but that their thermogenic response to food may be somewhat lower.

4. Two other observations that probably deserve mention at this time are:

A. The resting metabolic rates (expressed as ml O_2/min/kg FFM) do not appear to be significantly different for the Pima Indians than would be predicted from studies with lean or obese Caucasians.

B. Over-nutrition produces the same increase in resting metabolic rate in the Pima Indians as that found in similar studies with Caucasians in the Vermont laboratory.

Significance to Biomedical Research and the Program of the Institute: The Pima Indians are known to have a high incidence of obesity and Type II diabetes. Thus far, research concerning this population has focused primarily on diabetes, though the relationship between obesity and Type II diabetes has long been known. It is obviously important to the treatment and/or prevention of diabetes in the Pimas, as well as being important to its own prevention, to determine the cause(s) of obesity in this population. A group as genetically homogenous as the Pimas provides the best possible opportunity to find a genetic thermogenic defect, which would predispose an individual to obesity. If a defect does exist, it would then be of interest to determine whether or not such a defect exists in some Caucasians, or if the etiology of their obesity is due primarily to different mechanism(s).

Proposed Course: One additional volunteer is currently in the over-nutrition phase of the study and two are in the under-nutrition phase, to make a total of six for weight maintenance, six for over-nutrition and four for under-nutrition. These studies should be complete by the end of July, at which time we would like to statistically analyze the results. Analyses of the plasma and urine samples should be completed this summer as well.

It is planned that two related studies, which are currently awaiting approval and/or equipment, will be started in the fall. One of them is very similar in overall design to the one previously discussed, however, in place of the tests involving the thermogenic stimuli, the studies will include kinetic studies of T_3 and T_4 , TRH infusion studies (with measurement of TSH and prolactin response) and lipoprotein analyses. Since it has been suggested by the work of Flier and others that changes in the activity of the Na^+-K^+ATPase pump may play a role in the thermogenic response, the Na^+-K^+ATPase activity in red and white blood cells will also be measured while the subjects are in various nutritional states. The thyroid kinetic studies are important to the complete understanding of thyroid hormone metabolism in the Pimas and to the comparison of their thyroid hormone metabolism with that of Caucasians, which was studied previously in the Vermont laboratory. Na^+-K^+ATPase activity has recently been shown to have a negative correlation with body mass index in the Pimas. It is, therefore, of interest to try to determine whether this relationship is due to the actual differences in body weight and extent of obesity, or to the nutritional state of the individual.

The second phase of the proposed studies involves cold-adaptation of individuals by having them spend eight hours per day for five days per week for three weeks in a room kept at approximately 50°F. It has been reported that cold-adaptation causes Caucasians to become more sensitive to norepinephrine and that obese Caucasians have a decreased sensitivity to norepinephrine, when compared to their lean counterparts. Thus, it will be valuable to find out whether or not the Pimas are capable of adapting to the cold and what effect(s) prolonged cold exposure has on their sensitivity to norepinephrine.

This project is entirely supported under a subcontract with the University of Vermont.

Contract No. N01-AM-6-2219

Starting Date: September 1, 1980
Expiration Date: October 31, 1981

Contractor: University of Texas Health Science Center
Southwestern Medical School

Money Allocated: $2,794,619.

Man-Years Purchased: 32.2

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54030-01 DDB

PERIOD COVERED
October 1, 1980 to September 31, 1981

TITLE OF PROJECT (80 characters or less)

Radioimmunoassay of Polypeptide Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Vasquez	Postdoctoral Fellow	PCRS, NIAMDD
OTHER:	I. Klimes	Guest Worker	PCRS, NIAMDD

COOPERATING UNITS (if any)
Human Diabetes Study Center, University of Texas Health Science Center

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85016

TOTAL MANYEARS: 1.0	PROFESSIONAL: .2	OTHER: .8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The radioimmunoassay (RIA) laboratory supports epidemiological and metabolic studies which address the role of islet cell function and insulin resistance in the development of obesity and diabetes in the Pima population. RIA's for insulin, C-peptide, glucagon, and glucagon-like immunoreactivity (GLI) are performed in conjunction with several of the clinical studies and are used to investigate islet function and its regulation in obesity and diabetes.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The Pima Indians constitute a well-characterized population with a high prevalence of Type II diabetes mellitus and provide a unique population in which to study islet cell function, insulin resistance and the development of obesity and diabetes. Radioimmunoassays (RIA's) for measurement of circulating concentrations of pancreatic islet polypeptide hormones are central to these cross-sectional and longitudinal studies. In addition to the assays for insulinand glucagon, glucagon-like immunoreactivity (GLI) and C-peptide have been added in the past year.

Methods and Results:

1. Insulin and C-peptide:

A. Sacaton Field Studies Samples. Insulin is measured on a routine basis in serum samples obtained during biennial oral glucose tolerance tests on Pimas conducted by the Epidemiology and Field Studies Branch. Assays for insulin in samples collected over a six year period ending December, 1978 are completed and the data await statistical analysis.

B. Effect of Control of Plasma Glucose on β Cell function. Insulin secretion and insulin resistance were examined in seven obese Type II diabetics before and after control of plasma glucose levels without weight loss. Control was achieved by regular insulin injection (60-205 μU/day in four doses). After 10 days of therapy, plasma insulin and C-peptide responses to oral glucose were significantly improved. Insulin induced glucose disposal rates (M values), estimated by the glucose clamp technique were unchanged. In two diabetics, whose therapy was extended to 30 days, M values increased. These results indicate that short-term control of plasma glucose improved insulin secretion but not insulin sensitivity and that prolonged control improved both. The impaired insulin secretion and insulin sensitivity in Type II diabetics appears to be in part secondary to metabolic abnormalities associated with hyperglycemia.

C. Regulation of Islet Cell Function by Exogenous Insulin. To investigate the possible causes of hyperinsulinemia in obesity the C-peptide levels of 6 obese (OP) and 4 lean (LP) Pima Indians with normal glucose tolerance were studied using the euglycemic hyperinsulinemic clamp. On 3 different days plasma insulin was raised acutely 10, 25 or 100 μU/ml above its fasting concentration and maintained for 90 minutes. In the pre-clamp period insulin and C-peptide levels of OP were 3-fold greater than those of LP. Increases in plasma insulin of 10 and 25 μU/ml suppressed plasma C-peptide in both groups. An increment of 100 μU/ml suppressed C-peptide by 0.68 pM/ml (33% below baseline $P<0.01$) in OP and by 0.28 pM/ml (39% below baseline $P<0.02$) in LP. However, suppressed C-peptide levels of OP never reached the basal levels of LP. Termination of insulin infusions resulted in comparable rates of insulin disappearance. Glucagon was suppressed to a comparable degree in both groups. They reveal no evidence of an insensitivity of either β cells or α cells to regulation by insulin in the concentions studied.

2. Glucagon and GLI:

A. Lack of "Big Plasma Glucagon" Response to Arginine in Man. The glucagon antiserum 30K measures two components of immunoreactive glucagon (IRG), IRG^{3500} ("pancreatic glucagon") and BPG ("big plasma glucagon"). Although the BPG component constitutes the majority of the IRG in fasting plasma, a physiological role for BPG has not been demonstrated. Conflicting results have been reported from *in vivo* animal studies in which BPG response to arginine stimulation was attempted. In order to determine whether the concentration of "big plasma glucagon" (BPG) levels changes in response to stimulation of the pancreatic A-cell, plasma samples were obtained from eight Type II diabetics before and during arginine infusion. The BPG and pancreatic glucagon (IRG^{3500}) components of glucagon were separated by gel filtration chromatography and fractions were assayed for immunoreactive glucagon (IRG) with 30K antisera. When chromatograms from basal and stimulated plasma were compared, no changes in BPG were observed, demonstrating that only IRG^{3500} is involved in the increase in IRG observed in these patients.

B. Relationship of Fasting and Postprandial Plasma Glucagon and GLI to Obesity and Diabetes. Glucagon (IRG) and GLI as well as insulin concentrations were measured in plasma samples obtained before (-15, 0) and for 6 hours after (15, 30, 60, 120, 180, 240, 360 min) a standardized test meal was ingested by nondiabetic (NP, n=32) and diabetic (DP, n=12) Pima and nondiabetic (NC, n=11) Caucasians. All groups had similar means and ranges of obesity (body mass index). The fasting levels and integrated six-hour postprandial responses of insulin, glucagon and GLI were determined and the data for each of the three groups compared. The postprandial response of insulin was two-fold greater ($P<0.05$) in nondiabetic Pimas than in nondiabetic Caucasians. This difference is similar to previous reports from this unit demonstrating hyper-responsiveness of insulin in Pimas to oral and IV glucose when compared to Caucasians. The postprandial GLI response was also greater for Pimas (Mean PN response 160% of NC response; $P<0.05$), although the postprandial glucagon responses were not different. No significant differences between Pima nondiabetics and diabetics were observed in the postprandial responses of insulin, glucagon or GLI, although the time course of postprandial insulin response was more sluggish in DP than in NP and NC. The effect of BMI (obesity) upon basal hormone levels and postprandial responses was examined. Basal insulin correlated significantly with BMI for all three groups, as expected. Neither fasting nor postprandial IRG or GLI were related to BMI for any of the groups studied.

Significance to Biomedical Research and the Program of the Institute: The availability of accurate, well controlled assays for measurement of islet cell secretory products is essential to the investigation of the role of islet cell function and insulin resistance in obesity and diabetes. The RIA laboratory also provides the opportunity to develop methods and to approach more basic questions concerning the physiology of the polypeptide hormones.

Proposed Course: The RIA laboratory will continue to support studies by the Epidemiology and Field Studies Branch and the metabolic studies in this unit that require measurement of insulin, C-peptide, glucagon or GLI. In addition, we will attempt to develop a somatostatin in RIA that permits reproducible measurement of this islet hormone in unextracted plasma or in extracts of plasma of small enough volume to be practical for metabolic studies requiring multiple samples over a short period of time.

Publications:

1. Hidaka, H., Nagulesparan, M., Klimes, I., Sasaki, H., Aronoff, S.L., Vasquez, B., Rubenstein, A.H., and Unger, R.H.: Improvement of insulin secretion but not insulin resistance after short-term control of plasma glucose in obese Type II diabetics. J. Clin. Endocrinol. Metab., in press.

2. Von Schenck, H., Vasquez, B., and Unger, R.H.: Lack of a "Big Plasma Glucagon" response during arginine stimulation of glucagon secretion. Horm. and Metab. Res., in press.

This study is supported entirely by NIH N01-AM-6-2219.

Starting Date: September 1, 1980
Expiration Date: October 31, 1981

Contractor : University of Texas Health Science Center
Southwestern Medical School

Money Allocated: $2,794,619

Man Years Purchased: 32.2

ANNUAL REPORT FOR FISCAL 1981
OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL ACTIVITIES PROGRAM

The Extramural Activities Program (EAP) and the Office of the Associate Director (OAD-EAP) have responded to a wide variety of activities in FY 81. This reflects both the supportive and coordinating role of the OAD-EAP as well as the increasing number of responsibilities being placed upon it and its four branches: Grants Management, Contracts, Program Evaluation, and Review. The separate branch reports, which follow, detail accomplishments, challenges, changes, and new requirements as they pertain to the individual branches. This section refers to those activities emanating from the OAD-EAP and those having impact either across the branches of this cluster or across the extramural clusters and the NIADDK.

The NIADDK is completing its second year of participation in both the Division of Research Resources Minority Biomedical Support Program and the Short Term Training Program (T35s). The latter is authorized as National Research Service Awards.

A significant accomplishment during FY 81 has been the reorganization of the Administrative Office of the EAP. It is now staffed with three full-time people and has managed to clear-up a large backlog of vouchers, reports, and other administrative requirements long overdue. Further, it has assumed responsibility for management of our travel funds as part of our efforts to insure the accomplishment of all our cluster travel requirements.

The Associate Director together with key supervisory personnel in the cluster attended a Federal Executive Institute program on Organizational Development and Multi-Team Building. The group was enthusiastic in their response to the workshop and look forward to putting into practice some of the techniques learned.

An increasing emphasis is being felt on the coordinating function for this office. Increasing demands from the Office of the Director, NIH, the Office of the Director, NIADDK, and the program clusters have resulted in this office being used as a focus for collecting and disseminating information to and from the different groups with whom it coordinates Institute functions.

Two problems continue to affect the ability of this office and the EAP to respond effectively to Institute needs. Inadequate staffing continues to affect our performance, and the lack of space is a severe problem resulting in overcrowding. In some cases more than one person is assigned to a room and inadequate facilities for storage of records and essential documents continues to be a problem.

Program Evaluation Branch

The Branch provides information services to administrative and scientific officials regarding the NIADDK Programs, particularly the extramural programs. These services include but are not limited to analyses and evaluations of ongoing biomedical research programs and activities; maintaining a computer-based management information system (MIS); preparing special and recurring reports from its data base and from those of other MIS's; maintaining the NIADDK scientific thesaurus and scientifically indexing grant applications; assigning appropriate Program Areas to new grant applications; recording actions of the NADDK Advisory Councils and preparing the approval reports for each Council meeting.

This year we assigned 2400 new competing grant applications to the twenty four Program Areas, and scientifically coded over 4800 applications.

Requests for information, based on the scientific coding, represent about half the total special requests (currently over 250) answered throughout the year. A majority of these requests concern grant applications where NIADDK is the primary Institute. Therefore, we reduced scientific coding of dually assigned grant applications from a possible eight codes to two codes (primary and secondary), in each of the four axes. Also, progress reports of non-competing grant applications are scanned, rather than read in detail, for new biomedical information and discontinued research projects. If new directions are not evident and no project has ended, the scientific coding of the previous year is duplicated.

Results of these changes in coding procedures, for the 413 duals and the 1,962 non-competing grants, saved over 240 man-hours this year.

This year, as in the past, PEB reported and/or coordinated responses regarding Institute activities for several trans-NIH and national biomedical issues within the mission of NIADDK.

Examples of analyses and responses carried out during the year are summarized below, each is followed by the staff member's name who was responsible for its completion:

- The Federal Inventory of Population Research, 1980
  This annual report, of the NICHD, lists federally supported projects with relevance to population research including the reproductive process, contraceptives, reproductive behavior, fertility, sterility and reproductive endocrinology. The NIADDK supported 65 projects in this field for a total of 6.5 million dollars (up 6 projects and 1.6 million dollars from last year) of these projects, 60 were extramural grants and contracts for 5.2 million dollars and 5 were intramural projects for 1.3 million dollars. (Mr. Robert Petinga)

- Primary Disease Prevention, 1980
  Is a report of NIH projects concerned with primary prevention of disease or injury. We followed the guidelines from the OD, NIH and used the NIADDK scientific information system to formulate the Institute's response. More than 2,000 projects were evaluated and 161 designated as primary disease prevention research. All Institute programs are represented in the 161 projects which totaled 17.7 million dollars. (Mr. Robert Petinga)

- NIH Inventory of Clinical Trials, 1980
  The Inventory has not been requested by DRG for the 1980 clinical trials, as of now (6/30/81). A report of FY 1979 trials with future support was given to the FMO, allowing him to project estimates for fiscal years 1980, 1981 and 1982. (Miss Lois Reed & Mr. Robert Petinga)

- Alcohol Abuse and Alcoholism, 1980
  The NIADDK projects, related to this recurring annual report of research on alcohol abuse and alcoholism, were identified from the fiscal year 1980 scientific reports of PEB. Of the 10 projects identified with an estimated level of support of 0.4 million dollars, 9 are research grants and 1 is an intramural project. (Ms. Linda Stalvey)

- Marquis Academic Media, 1980
  PEB coordinated the extramural programs' response to Marquis Academic Media's annual request for funding information. In previous years funding was reported by cluster in some instances, and program area in others. To synchronize FY 80 reports PEB:
  1) defined the 10 major program areas: Arthritis, Musculoskeletal Diseases, Skin Diseases, Diabetes, Endocrinology, Metabolic Diseases, Digestive Diseases, Nutrition, Hematology and Kidney Diseases.
  2) asked program directors to provide a written description of their area of scientific interest, or to approve last year's description
  3) used PEB's end of year reports to provide monies spent in each category
  4) defined the categories as follows:
     a) Research: includes K's, R's, and P's.
     b) NRSA: includes F's and T's (T35's excluded)
     c) Contracts (N's)

  This procedure should simplify collecting data for Marquis Academic Media, or other similar organizations wanting this information. (Ms. Linda Stalvey)

- Systemic Lupus Erythematosus (SLE)
  Is an inventory of currently active (January 1981), NIH supported research, whose response was coordinated by NIADDK. Both CRISP and each B/I/D's scientific information systems were used as data bases. The total NIH support in SLE research was 151 projects for 8.7 million dollars. NIADDK's portion was 80 projects for 5.5 million dollars. (Mr. Robert Petinga)

- Benign Prostatic Hyperplasia (BPH) 1979, 1980

  At the request of the Associate Director of the KDUB Cluster, the possible trans-NIH support of BPH for FY's 1979 and 1980 was retrieved from both the CRISP and Institute information systems. The results of our analysis of the abstracts and coding terms are displayed below. (Mr. Cecil Lee)

Support Of BPH Research

Institutes	1979	1980
NIADDK	$671,146	$766,907
NIA	40,263	101,725
NICHD	45,791	43,340
NCI	----	65,599
Total NIH	$757,200	$977,571

- Viral Hepatitis, 1980

  Request from the Hepatitis Research Director, NIAID for the U.S. - Japan Cooperative Medical Science Program for NIADDK support of viral hepatitis research. Totals are percent of viral research times the total dollars for each project. The Institute reported:

	8 Extramural grants for	$349,729
	3 Intramural projects for	402,500
Total	11	752,229

The Program Analysis (PA) Section, PEB is working with the Digestive Diseases Coordinating Committee (DDCC) to develop a system for tracking digestive diseases research in the NIH community and in other federal agencies. PA is assisting the group in understanding how the IMPAC, CRISP and NIADDK coding systems operate and interact. Also, we are identifying special interest areas in the Institute and throughout NIH that are not easily identified in current NIH systems, but are of interest to many private and government groups. The DDCC is meeting regularly and we should have a digestive diseases tracking system in operation by the end of the fiscal year. (Mr. Harry Free)

The Branch gave two presentations to the NAMDD Advisory Council at their January, 1981 meeting.

One was an analysis of M.D. and Ph.D. applicants and awardees from 1970 through 1980. While the number of M.D. trainees remained stable the number of Ph.D. trainees increased. This same trend was observed for regular research grant applicants, especially between 1977 and 1980. The increase in Ph.D. applicants did not affect the proportion of M.D. vs Ph.D. awardees, which remained relatively constant from 1970 through 1980. The strength and number of NIAMDD's M.D. researchers was in contrast to the NIH trend, which shows a decreasing number of M.D. awardees. (Mr. Robert Petinga)

The second presentation summarized the 10 year history of foreign funding by the NIAMDD and NIH, of traditional research grants (R01). Table I gives the percentage of foreign awards for the Institute and NIH. Table II displays foreign awards by Program Area. Table III reviews foreign funding in the U.S. - Japan Program. (Mr. Larry Greene and Miss Lois Reed)

Percentage of Foreign Awards

Table I

(R01's)

NIAMDD and NIH

	% Foreign Numbers		% Foreign Dollars	
FY	NIAMDD	NIH	NIAMDD	NIH
1971	0.9	1.2	0.7	0.8
1972	1.1	1.0	0.7	0.6
1973	1.0	1.0	0.7	0.7
1974	0.7	0.9	0.5	0.7
1975	0.9	1.0	0.6	0.7
1976	0.6	0.8	0.5	0.7
1977	1.0	1.1	0.7	0.9
1978	1.2	1.1	1.0	0.9
1979	1.4	1.2	1.3	1.1
1980	1.5	1.2	1.3	1.1
Average	1.0	1.1	0.8	0.9

FOREIGN AWARDS (R01's)

Table II

Direct Cost Dollars (In Thousands)

by Program

FY	KD	HEM	DD	NUTR	DIA	ENDO	META	NIAMDD
1971	27	56	45	18	-	63	147	356
1972	30	45	40	15	6	65	229	430
1973	-	85	87	14	-	58	159	403 (438)*
1974	-	71	18	27	-	61	154	331 (365)
1975	-	57	114	29	-	57	180	437 (473)
1976	-	48	116	69	-	20	221	474
1977	25	20	30	88	139	94	236	632
1978	23	110	54	81	473	134	146	1021
1979	61	77	90	47	987	131	232	1625
1980	120	66	89	-	1002	80	410	1767 (1805)

* () Include the few foreign awards made in the AMSD Programs.

Table III

US - Japan Program (Foreign Awards Only)

(Dollars in Thousands)

	AI		AM		HD		NIH	
FY	NO.	Dollars	NO.	Dollars	NO.	Dollars	NO.	Dollars
1971	--	--	(6)	$ 174	(2)	$ 42	(8)	$ 216
1972	(4)	$ 46	(6)	135	(3)	87	(13)	268
1973	(2)	21	(4)	156	(2)	57	(8)	234
1974	(2)	18	(5)	116	(2)	54	(9)	188
1975	(1)	15	(1)	33	(1)	22	(3)	70
1976	(1)	10	(1)	52	(1)	30	(3)	92
1977	(1)	10	(1)	52	--	--	(2)	62
1978	--	--	(1)	54	--	--	(1)	54
1979	--	--	(1)	20	(1)	80	(2)	100
1980	(1)	7	--	--	(1)	71	(2)	78
Total	(12)	127	(26)	792	(13)	443	(51)	1,362

The Branch, particularly the Information Storage and Retrieval Section, produced several special reports that have become recurring.

One consists of summary tabulations of the Institute's fiscal year grant obligations produced in the format needed by the Financial Management Office.

Another major report is a year to date report of competing applications and awards in priority order listing cumulative dollars. This report is sorted in three different ways: by Institute, by major program; and by cluster. In each report, regular research (P01, R01, R22, R23) is combined, and other activities are listed separately. This recurring report is now produced pre- and post-council, and at the end of the fiscal year.

Also, PEB is working with staff of the Diabetes Mellitus Coordinating Committees to more fully automate the input of their categorical codes, and production of reports.

PEB continues to provide information services to Institute staff and others, as priorities and resources allow. We are pleased with the accomplishments of the Branch but disappointed that lack of resources (particularly personnel and space) have not allowed PEB to increase its utilization of current technological advances in the computer and information fields. With little expectation of relief, PEB's services must continue near present levels with minimal expansion in identified needed directions.

CONTRACT MANAGEMENT BRANCH

Over a dozen competitive procurement actions were initiated during the fiscal year. Responses to our formal requests for proposals ranged from two or three to fifteen or more. Offerors who submit proposals are usually well informed about the regulations concerning competitive range negotiations; and are also keenly aware of debriefing procedures and Freedom of Information Act prerogatives. Seldom is an award now made without being challenged by several other interested third parties.

At the Departmental level, attention is focused on the following competitive and scheduling goals to insure that:

- ° Competitive awards represent at least 53% of total annual obligations; and
- ° Fourth quarter awards do not exceed 30% of total annual obligations.

Administrative closeout of expired contracts is progressing in accordance with the Department's mandates; monitoring continues with complex quarterly closeout reports. Although our delinquency list of overdue final reports has shrunk, prompt submission of final reports by all contractors has not been achieved.

Another key negotiator left during the year due to lack of promotional opportunity. No replacement was hired. Instead, the remaining staff was reorganized. As a result, better support is being provided to the most active program area. A secretary is still needed; having one would help the work flow and yield more productive time to other staff members.

However, adequate accomodation has not been obtained for existing employees. Office productivity has been improved by acquisition of a Lexitron, which helps us cope with constantly evolving "boilerplate" and other fundamentally repetitive operations.

Direct audit activity has diminished in contrast to previous years, but it is evident that both Congress and the Department retain interest in the accountability aspects of the entire procurement process. Therefore, greater effort has been made to meet the following objectives:

1. Prevent the use of irresponsible audit techniques by outside review teams;
2. Resolve disputes with offerors and incumbent contractors in a reasonable time frame;
3. Eliminate communication barriers and minimize procurement policy and procedure differences with program personnel;
4. Improve the working relationship between the NIADDK contract office and the NIH Board of Contract Awards (BCA).

In regard to item four above, the threshold for BCA pre-award review was raised from $150,000 to $250,000. This long overdue action alleviates an aggravating logistical bottleneck. A parallel threshold is needed for noncompetitive concurrence and approval procedures.

Training of Institute project officers in accord with HHS requirements has been achieved; no temporary waivers were forwarded to higher echelon for countersignature. Each negotiator has either been certified at an appropriate level or submitted an application for certification. Although training and/or certification requirements are in a state of flux, a repetition of previous deadline-type approaches is not expected.

Socioeconomic issues - especially the small and disadvantaged subcontracting program - have proven cumbersome to negotiate and administer. Although Public Law 95-507 applies only to awards of $500,000 or more, reporting requirements are onerous to contractors. Therefore, voluntary compliance is erratic. Since socioeconomic goals are not always realistic at inception and the statistical feedback from contractors is not dependable, only a marginal possibility exists that they can all be met.

Finally, perhaps the foregoing conveys the impression that contracting has become complicated. It has, but the mechanism is not expected to self-destruct. We are therefore committed to providing more insight into the procedural aspects to Institute Staff who are involved. To this end, conferences were held with each Associate Director of the respective clusters. More meetings are encouraged with program managers/project officers as circumstances dictate. Only with a cooperative effort can lead time be cut, procurement planning be effected, competitive/noncompetitive balances be maintained and award dates be distributed throughout the fiscal year.

Grants Management Branch Annual Report

This year, the NIH has begun to implement a significant improvement in the awarding and administration of research grants in support of projects that involve multiple B/I/D interests. The concept of Co-Funding has been discussed for several years; but this year has seen the first actual awards. It appears that increasing interest in trans - NIH issues has brought about this change. Grants Management across the NIH has provided the resources necessary to produce the implementing documents. So far, this concept has been used to award certain Conference grants as well as our contribution to Minority Biomedical support grants. This new procedure allows several B/I/D's to contribute partial funding of an individual award through the use of separate Common Accounting numbers (CAN) for each Institute appearing on the Notice of Grant Award. This is accomplished by a memo detailing the agreement of all parties (within NIH) to the level of funds that each will contribute and to which institute will administer the award. The memo includes a sign-off by Grants Management Officers authorizing the use of the CAN numbers and supporting funds. Each such Cofunded award gives recoginization to the B/I/D's that have assisted in that award.

A decision announced last year by the Office of Management and Budget (OMB) is now beginning to have significant impact on our ability to manage our awards. That is, we can no longer request grantees to routinely send in their annual Reports of Expenditures (ROE's) showing budget category breakdowns. Previously, we could compare such things as personnel expenditures with personnel funds awarded and equipment expenditures with equipment funds awarded. Initially grantee institutions continued to submit categorical ROE's. However, we are now receiving less than 50% of ROE's showing expenditures by direct cost category. Consequently, we are rapidly losing our ability to track the fiscal and administrative performance of our grantees. When reviewing requests for approval of carryover of funds or of administrative increases, it is becoming impossible to determine the history of how a given grant has utilized its funds. Fortunately, we are allowed, in special cases, to request the categorical breakdown. We have continued to exercise this option for our major center (Arthritis and Diabetes) awards. From all appearances, grantee computerized accounting system are still generating their own reports in categorical detail.

The struggle over the NIH's training programs (National Research Service Awards - NRSA) has continued through this year. Once again, forgetting for the moment the serious impact of this struggle on the academic community and on trainees and fellows, an extra burden has been put on the Grants Management Branch. This year the debate raged over the propriety of providing institution allowances and indirect cost on our NRSA awards. At one point our allocation of operating funds for NRSA was reduced by an amount of almost 7 million dollars to eliminate those portions of the awards. The NIH provided interim operating instructions detailing the method of issuing NRSA awards on this basis. We had no sooner begun to issue these altered awards when it became clear that another change in policy was imminent. Rather than continuing to issue incomplete awards, we placed an unofficial "hold" on the issuance of further NRSA awards. While this eliminated the double work of having to revise large numbers of awards, it created considerable havoc in our attempt to realize our goal of the timely issuance of awards.

However, a positive change was implemented during the year in an attempt to help resolve our problem with the timely issuance of competitive awards. This has been a problem because of our inability to consistently issue New and Competing Extension awards for their scheduled begin dates, especially in view of NIH's requirement for prospective begin dates on competing awards. Clearly, some of the causes of this problem, such as being on a continuing resolution or being with out a current allocation of funds to program areas and mechanisms of support. are beyond our ability to control. However, in spite of these problems, it is evident that we can initiate a conservative payment of applications immediately after Council and before submission of formal pay plans by individual program areas. We feel confident that with proper discussion of this earlier processing of competitive awards with program staff, we will be able to provide more timely awards.

As usual, our major concern within the Grants Management Branch is with our most valuable resource, our own personnel. It cannot be stressed too much, that it has only been through the diligent and dedicated effort of our staff, that we have been able to maintain the efficiency of the awarding process. It is of deep concern, however, that this efficiency in the awarding process may have detracted from the recognition of our role in the post award management of grants. It is this area that the potential exists for the Institute to be judged deficient. During the year, statistical information depicting both NIH wide grants management staffing patterns and award patterns became available. Comparision of this Institute with other institutes provides solid evidence that proportionally, significantly more manpower is allocated to grants management functions in other institutes than in the NIADDK. Given this distribution of manpower, the Grants Management Branch has been very successful in the awarding of our inventory of grants and in the execution of the extramural budget. However, in the current climate of "accountability" within government, it is of concern that the Institute may not have allocated sufficient resources to the post award business management of our granting activities.

Howard P. Morgan
for John R. Garthune
GMO, EAP-NIADDK

ANNUAL REPORT FOR FISCAL 1981
REVIEW UNIT, EXTRAMURAL ACTIVITIES PROGRAM
NATIONAL INSTITUTE OF ARTHRITIS, DIABETES AND DIGESTIVE AND KIDNEY DISEASES

The Review Unit is now rsponsible for the technical merit review of applications for training grants, cooperative clinical studies, clinical investigator awards, program projects, core center grants, comprehensive center grants, and cooperative agreements.

These applications reflect the broad spectrum of NIH activities concentrated within the Review Unit for the specialized program efforts of the broadly-based four disease-oriented program clusters of the NIADDK. More than 50 committees were required to undertake the technical merit evaluation of the proposals for this fiscal year.

Although the total number of applications under review has been quite large for our resources, the present review staff has been able to provide quality merit reviews through use of the flexibility inherent in the ad hoc review mechanism. The 194 grant proposals in Table 1 reflect a total request by applicants of 167 million dollars. Evaluation of these proposals by our consultants resulted in recommendations of less than 50 percent of the amount requested (77 million dollars) as appropriate in making funding decisions. In the case of the contract proposals, a total of 6.4 million dollars was requested. Consultants recommended that approximately 50 percent (3.6 million dollars) be considered appropriate for negotiations and subsequent funding decisions.

TABLE 1. NUMBER OF PROPOSALS REVIEWED BY REVIEW UNIT FOR FY 1981

	COUNCIL			
Activity Code*	Oct. '80	Jan. '81	May '81	Total
P01	9	9	0	18
K08	0	13	24	37
T32	19	17	32	68
P30, P60	8	2	7	17
N01	25(5)	2(2)	10(3)	37(10)
Other	2	4	11	17
TOTAL	63	47	84	194

*P01 = Program Project
T32 = Institutional Fellowship
K08 = Clinical Investigator
P30 = Core Center
P60 = Comprehensive Center
N01 = Contract Proposals (RFPs)

Two standing peer review groups for handling manpower and research applications have been proposed. One (AMRA) has been formally constituted and the other (AMRB) is in progress. A request for establishment by charter is contemplated upon completion of our present structuring efforts. The Special Projects Review Committee for which a charter had been requested has been approved. This committee is comprised of three sections (subcommittees) and formal appointment of consultants to each of these sections is in progress. Section A is essentially in place and functioning for the review of multipurpose arthritis centers. Sections B and C are in the process of being activated.

The Review Unit is comprised of 12 people, including six professional members. It is estimated that the overall workload exceeds our resources by 30 percent. The readily accessible Savin model copy machine, flexible utilization of grants technical assistants, and the willingness of staff to cooperate have all contributed to our success in making deadlines. However, continuation of reviews without additional staff is unlikely to provide increased quality of reviews. The present efforts to develop additional standing committees will further strain our present resources. The magnitude and variability of the workload, the constraints of time imposed by council rounds and establishment of standing committees have made critical the need for an additional grants clerk. Such a clerk could have a significant impact upon our capability to meet our deadlines by being a flexible resource to be assigned temporarily to review groups for extra assistance to meet a specific crisis. This grants clerk also would be able to substitute for one of those in the review sections should illness or change in employment occur. Further, such a substitution needs to occur with a knowledgeable grants clerk familiar with the operation of the Review Unit. This can readily be accomplished through recruitment at a GS-3 or 4 level for a clerk typist who would be assigned to the office of the Chief of the Review Unit to function in this role, and to also assume the responsibilities of timekeeper or disbursing pay check agent. The assumption of these and other duties by this grants clerk would permit a greater role to be played by the grants technical assistant presently in this office for assuring quality document preparation and timely document flow by the grants assistants within the Review Unit. An additional position needs to be allocated to the Review Unit for this purpose.

Although training outside of the Review Unit is available and staff is encouraged to take advantage of it, the workload staff ratio imposes severe constraints upon this type of activity.

DIABETES, ENDOCRINOLOGY, AND METABOLIC DISEASES CLUSTER

OFFICE OF THE ASSOCIATE DIRECTOR

The Associate Director plans and directs programs of research grants and contracts, training and fellowship awards, and special programs and activities within the Diabetes, Endocrinology, and Metabolic Diseases Cluster. The Associate Director is responsible not only for strengthening, integrating, and coordinating activities within these areas but also for developing and initiating new programs to meet the needs of the scientific community.

In the area of diabetes, the Associate Director, acting through the NIH Diabetes Coordinating Committee, has primary responsibility for the management of all trans-NIH activities relating to diabetes mellitus, assuring better coordination, a sharper focus for program activity, and accountability in diabetes among Institutes.

The Associate Director also serves as Chairman of the Inter-Agency Diabetes Mellitus Coordinating Committee, whose membership includes representatives from all federal agencies with an interest in diabetes mellitus. He plays a lead role as liaison to voluntary health groups and scientific societies concerned with diabetes, endocrinology, and metabolic diseases. Thus, he is frequently and intimately involved with the activities of such groups as the American Diabetes Association, the Juvenile Diabetes Foundation, the Cystic Fibrosis Foundation, and the Endocrine Society.

The Associate Director also directs special activities concerned with diabetes, endocrinology, and metabolism. He has directed the development of a plan to study, through a clinical trial, the effect of strict blood glucose control on the complications of diabetes. A Request for Applications to conduct such a study under the Cooperative Agreement Mechanism was developed and issued during this year as was a Request for Proposals for a Data Coordinating Center. The study will begin in 1981. The Associate Director participates in the planning and monitoring of the Institute's research activities in the Clinical Research Center at the Epidemiology and Field Studies Branch in Phoenix. The Associate Director actively works toward fostering and enhancing more and better NIADDK-grantee institution-private sector relationships through frequent meetings, on site visits and formal and informal discussions.

In addition to his programmatic responsibilities and coordinating activities, the Associate Director carries on his own program of research. His laboratory is investigating the relationship between diabetes and obesity with a particular focus on the adipose cell as a model for the study of insulin's regulation of glucose metabolism.

DIABETES PROGRAMS BRANCH

This office coordinates the activities of the Diabetes Research Program, Diabetes Centers Program, National Diabetes Data Group, National Diabetes Information Clearinghouse, and the new Clinical Trial Program. In addition, it provides an administrative focus for liaison regarding training, career, and resource development activities in diabetes and related areas of endocrinology and metabolism. It also coordinates support for diabetes-related awards in other NIADDK programs and for diabetes-related workshops, conferences, and symposia sponsored by the Institute. The highlights of these activities are summarized, as appropriate, in the following sections of this report. Although not detailed below, the staff of the Diabetes Programs Branch also serves as an information resource to the scientific community, lay public, and other Federal, state, and private organizations with respect to the full spectrum of national diabetes research, training, and information-transfer activities. In addition, the staff also participates in and contributes to a wide variety of NIH-sponsored activities which, directly or indirectly, support the continued development of expanded scientific capabilities both in diabetes and other areas of biomedical research.

Diabetes Research

During the past year, progress has continued in a wide range of scientific disciplines which are fundamental to research on diabetes mellitus and its complications. These studies focus primarily on defining the nature, etiology, epidemiology, and pathogenesis of diabetes and its complications in a long term effort to develop improved medical capabilities for prevention, diagnosis, treatment, and cure of this disease. Within this context, some areas of particular research interest include studies involving islet cell transplantation; insulin delivery systems; the epidemiology of diabetes and its complications; the biosynthesis, secretion, action, interaction, and degradation of insulin and other related hormones; the physiology and pathophysiology of pancreatic islet cells; abnormalities of carbohydrate, lipid, and protein metabolism associated with diabetes and its complications; the possible relationship between various genetic, viral, immunologic, toxicologic, and nutritional parameters and the etiology of diabetes and its complications; and biobehavioral research related to the psychosocial aspects of diabetes. Recent progress in some of these areas is summarized below:

Islet Transplantation. Transplants of islet tissue within inbred strains of animals have been shown to successfully reverse experimentally induced diabetes mellitus and to prevent or reverse early diabetic microvascular complications. New techniques have been developed which can prolong the survival of rat islet allographs across major histocompatability barriers by procedures which do not require continued administration of immunosuppressive agents. However, in order that islet cell transplantation can be successfully evaluated in humans, it will be necessary to further define the mechanism of immune rejection. It has been shown in rats that had received successful allographs, that rejection could be induced by injection of peritoneal exudate cells from the original donor. The exudate cells that cause the rejection contained more than 50 percent macrophages and T-lymphocytes. B lymphocytes do not induce rejection of established allographs. Identification of the specific types of lymphoid cells required for the induction of rejection may make it possible to utilize specific antibodies for destruction of these cells in the islets before transplantation. These studies indicate that islet cells are incapable of initiating their own rejection yet will serve as a target for an established immune response. This suggests that certain components of the major histocompatibility complex may not be expressed in islet cells. In this regard, recent studies have shown that islets cells from three different mouse strains express products of the

H-2K and H-2D loci but do not express I region (Ia) antigens. These findings could explain why the established allographs do not stimulate their own rejection (lack of the Ia antigen) and why the cells can be recognized targets (presence of H-2K and H-2D antigens) when immune recognition is initiated by donor lymphocyte cells.

Insulin Delivery Systems. During the past year, the Program continued to support the development and evaluation of both portable and implantable automated insulin delivery systems for use in biomedical research. Recent progress in this area has been encouraging, and suggests that such devices may offer the potential for improving the control of blood glucose in insulin dependent diabetes during the course of long-term outpatient studies. For this reason, it is now anticipated that open-loop insulin delivery systems will be used in the Program's recently-announced multicenter clinical trial to assess the relationship between blood glucose control and prevention, arrest, or amelioration of the early vascular complications of diabetes.

As suggested above, it appears that the Program's contracts for the development and refinement of automated insulin delivery systems will soon meet their stated goals, and that a new generation of reasonably sophisticated open-loop devices will soon be a reality. Although none of these systems will represent the ultimate that modern technology could provide, it is anticipated that they will be adequate to assess whether or not further refinement might be appropriate. In addition, new developments in the private sector now suggest that a variety of automated insulin delivery systems will be marketed and made available in the not-too-distant future. For these and related reasons, the Program's contract support for the development of new open-loop insulin delivery systems will be phased out over the next several years as the projects which are now underway each move to completion. Nevertheless, support for new studies in this area will remain available under the investigator-initiated research grant mechanism.

Hormone Action. In past annual reports, reference has been made to various studies on the mechanism of insulin action. It is now known that insulin reacts with cell surface receptors and that the insulin-receptor complex is somehow internalized resulting in the association of insulin and intracellular particles. Several enzymes are regulated by insulin in this process. The activities of the enzymes in some cases involve phosphorylation and de-phosphorylation reactions. Various candidates have been proposed for mediating the effects of insulin and evidence has accumulated for a "mediator" substance which has been reported previously as acting in two systems and which was involved in the activation of glycogen synthase phosphotase and pyruvic dehydrogenase. Recently a cell-free system has been developed using plasma membrane and mitochondria from rat adipocytes in which insulin acts to decrease phosphorylation of the mitochondrial enzyme pyruvate dehydrogenase which results in increasing its activity. The mechanism of action involves a "mediator" substance. This same cell-free system has been used to demonstrate another step in the action of insulin subsequent to its binding. Activation of a membrane protease which is stimulated by the binding of insulin and results in the production of a soluble factor (mediator) that eventually stimulates the pyruvate dehydrogenase activity when added to mitochondria. Concanavalin A or antibody to the receptor can mimic the ability of insulin to activate pyruvate dehydrogenase. Recent evidence also suggests the presence of an anti-mediator in the cell.

The activation of glycogen synthase by insulin has also been demonstrated in human skin fibroblasts, a system which could offer the potential of a genetic marker for the diabetic state.

A primary effect of insulin on adipose and muscle cell function is the stimulation of glucose transport activity. Kinetic studies support the concept that insulin stimulates transport by increasing the maximum velocity of the transport system. However, an effect of insulin on the mobility of a fixed number of transport systems versus an effect on the number of these systems cannot be distinguished by this type of study. Recent studies measuring the binding of [^{3}H] cytochalasin B to the plasma membrane-rich and microsome-rich preparations of adipocytes in the presence and absence of high concentrations of D-glucose by one group and the solubilization of glucose transport activity, reconstituted and placed in egg lecithin liposomes in order to measure D-glucose uptake, by another group, indicate that, insulin stimulates glucose transport in intact cells, primarily by increasing the number of these systems.

Insulin resistance is a characteristic feature of both obesity and non-insulin-dependent diabetes mellitus. The resistance can result from either an abnormal beta cell secretory product, circulating insulin antagonists or target tissues with defects in insulin action. Present evidence suggests that insulin initially binds to surface receptors, forming an insulin-receptor complex which is processed in some way and internalized. Subsequently the insulin is degraded, probably after associating with lysosomes. Recent studies from one group suggest that lysosomal function is necessary to mediate any subsequent degradation of internalized receptor and that without this degradation, receptors may be recycled. Another group, based on their recent work, suggest that insulin receptors may be able to shuttle back and forth between plasma membrane and intracellular sites of insulin degradation without loss of receptor caused by receptor-mediated insulin degradation.

Hormone Synthesis. The synthesis of hormones using recombinant DNA techniques is still an active research area of interest. New insight into the evolutionary clock has recently been provided by using recombinant DNA techniques to study the molecular basis for the biosynthesis of two rat preproinsulins as well as chicken preproinsulin. Researchers found two non-allelic genes in the rat that differed by having one (Rat gene I) or two (Rat gene II) intervening sequences (introns). They examined the chicken preproinsulin gene and found that the gene was present in only one copy and contains two introns similar to the rat gene II. They concluded that introns can be lost during evolution and that they must play a role in the assembly of genes. The molecular analysis of the preproinsulin genes in normal and diabetic animals could contribute important background information on the pancreatic B-cell dysfunction.

The human insulin gene was sequenced and was also found to be similar to the rat gene II. Evidence is also emerging that there are two alleles of a single insulin gene. Allelic differences have been observed in the 3'-untranslated region of the human insulin mRNA and it has been suggested that this is a form of polymorphism which could affect insulin biosynthesis.

A hyperinsulinemic patient was recently identified who responded normally to exogenous insulin. The insulin from the patients' serum had lower than normal binding and biological activity. A piece of the patients' pancreas was obtained during exploratory surgery and the insulin contained in the beta cells was found to be a mixture of a normal and a variant insulin which contained a leucine for phenylalanine substitution at position B24 or B25. This was taken as evidence for codominant expression of 2 allelic genes in the heterozygous state. Further studies separated the patients two insulins into one with normal activity and one with low activity which in turn antagonized the action of the normal insulin. Somatostatin, a tetradecapeptide originally isolated from the hypothalamus and subsequently located in the pancreatic islet D cell, has been suggested as a local regulator of insulin and glucagon secretion and may be involved in the pathogenesis of diabetes. Most of the studies to date have been performed on the fourteen amino acid peptide originally isolated, but recently investigators have isolated a longer twenty-eight amino acid somatostatin. This peptide is ten times more active than the smaller peptide and the suggestion is that this is the "real" somatostatin and that the fourteen amino acid peptide is a degradation product of this compound.

Diabetogenic Agents. A recent study looked at the effects of the oral sulfonylurea agent glyburide to modulate insulin receptors on non-transformed human fibroblasts in culture. Insulin can induce a considerable loss of insulin receptors in these cells. However, when the process of insulin-induced receptor loss or down regulation was studied in the presence of glyburide, the drug inhibited the insulin-induced receptor loss in a dose dependent fashion. When glyburide was added to the fibroblasts in culture, a dose dependent increase in the number of cell surface receptors was observed. These studies demonstrate a direct *in vitro* effect of the oral hypoglycemic agent to increase the number of cell surface insulin receptors and prevent the loss in the presence of excess insulin. This helps to explain the well-known extra pancreatic effect of the sulfonylurea agents in improving insulin-mediated glucose metabolism.

Another oral hypoglycemic drug, pirogliride has recently been shown to accelerate glucose usage and insulin secretion by the pancreatic islets. This drug could be useful in probing the underlying mechanism of stimulation of insulin secretion and also as a therapeutic agent for those diabetics who have impaired B cell responsiveness.

Complications. The kidney is a target for the secondary microvascular complications of diabetes. Evidence seems to suggest that altered microvascular hemodynamics caused by the diabetic state contribute to glomerular pathology in some unknown way. The glomerular pathology includes glomerular basement membrane thickening and mesangial thickening. There is evidence that the mesangial thickening is reversible by ameliorating the diabetic symptoms but that the glomerular basement membrane thickening is not. It is estimated that 50% of children with IDDM will develop renal failure within an average of 20 years after onset. After a hiatus of several years, pancreas/islet cell transplantation is being explored again for the diabetic uremic patient. Initial results of partial pancreas transplantation in patients stabilized for one or two years by successful renal transplantation seem promising.

Improved understanding of the pathogenesis of diabetes could contribute to the amelioration or prevention of these complications. In order to stimulate additional interest in this important area of research, the NIADDK recently issued a Program Announcement soliciting research grant applications which propose studies of the kidney related to diabetes mellitus.

Immunological Factor and Genetics. Results of various studies are beginning to unravel the immunogenicity of pancreatic islets. Monoclonal antibodies, advances in purification of membrane components and a better understanding of the immune system should combine to permit elucidation of the role of alterations in immune response in the genesis of insulin-dependent diabetes mellitus (IDDM). Early experiments in animal models suggest that it may be possible to design immune interventions to arrest the process in the early development of IDDM and thus sustain some pancreatic islet function.

In the past decade it has become apparent that the immune response is precisely regulated and the regulation operates at several levels. This regulation is very complex and involves the products of a linked set of genes that determine the structure of the major transplantation or histocompatibility antigens (HLA antigens). The antigens are found on all nucleated cells and platelets. Susceptibility to various diseases has been found to be associated with them, particularly at the HLA - A, B or D locus. The mechanisms of that association are unknown. In order to stimulate interest in this area, to review the available science data base and to allow for interaction between diabetologists and immunologists, the NIADDK in conjunction with NIAID and NIDR sponsored a workshop in November 1980 on the Initial Lesion of IDDM. After reviewing and discussing all of the available data, the following future needs were identified:

1. more standardized epidemiologic data in the U.S., including incidence and prevalence of IDDM;

2. pooling of data in the U.S. relative to HLA typing, possibly requiring a centralized facility;

3. standardization of testing for islet cell antibodies (ICA's) and islet cell surface antibodies (ICSA's) and assessment of their roles in the pathogenesis;

4. better morphologic data to characterize the nature of the round cell invasion of the islets;

5. need for microcytotoxic assays to follow the levels of cellular and circulating immune responses to the B-cells; and

6. a clearer image of the present natural history of diabetes using current therapeutic principles and methods to determine the cost/effectiveness/benefit of any proposed intervention.

Recent investigations have shown that genetically obese (Ob) mice have improved metabolic efficiency. These mutant mice gain more weight on the same amount of food than do normal mice, and upon fasting are able to utilize their stored resources in a more efficient fashion. When gaining weight, Ob mice store most of their reserves as fat. On fasting, Ob mice survive 5-8 times longer than normal mice and analyses have shown that they are capable of utilizing all their stored reserves to prolong survival.

In vitro experiments have shown that acetone, normally an end product of lipid degradation, can be converted to lactate in mice through a sequence of as yet undefined steps. Lactose in turn can be converted to glucose which can fuel necessary metabolic activities. This recycling of potential waste products may permit the complete utilization of fat stores and greater survival time during fasting. This "thrifty" mutation may provide a selective advantage during times of food deprivation. However, in times of stabilized food sources, this "thriftiness" becomes deleterious, as over-eating and obesity strain the insulin synthesizing capacity of the B cells of the islets.

Clinical Trial in Insulin-Dependent Diabetes Mellitus

Within the last year, plans have been formulated for a multicenter randomized clinical trial to assess the relationship between blood glucose control and the early vascular complications of insulin-dependent diabetes control and mellitus. In early 1981, a Request for Research Cooperative Agreement Applications (RFA NIAMDD 81-01) and a Request for Proposals (RFP NIAMDD 81-9) were issued to identify, respectively, clinical centers and a data coordinating center interested in participating in such a trial. Several hundred requests for the RFA and RFP were received. Sixty-six responses to the RFA and 14 responses to the RFP were submitted, and these are currently undergoing peer review. It is anticipated that approximately twenty clinical centers and one data coordinating center will be selected for participation. They will be activated by December 1, 1981.

The overall objective of this clinical trial is to determine whether and to what extent tight control of blood glucose (intensive therapy) is effective in preventing or ameliorating the early vascular complications of this disease. Much of the framework for this trial has been developed around the recommendations of the ad hoc consultant group noted in last year's Annual Report. It was specifically recommended by this expert group that participants between the ages of 10 - 23 with insulin-dependent diabetes of less than 10 years' duration be randomly assigned to either an intensive or a conventional therapeutic regimen, that the intensive regimen utilize the recently developed technologies for blood gulcose control such as automated insulin delivery systems and instruments for home blood glucose monitoring, and that the occurrence of selected pathologic complications in the two groups be compared with particular attention directed to retinal changes. These recommendations have been used as the structural bases in developing plans for this trial.

It is anticipated that the trial will consist of four sequential phases (planning, limited feasibility testing, full-scale clinical trial, and data analysis/reporting). A complete evaluation of the experience and results of each phase will be performed prior to intiating the next scheduled phase to determine whether it will be implemented and how it is to be undertaken. A Policy and Procedures Advisory Group of expert consultants who are both independent of the trial and external to the Institute will continually review all actions which affect the broad organizational, operational, ethical, and design features of the trial. Approval by this group will be required for proceeding with the initiation of each new phase.

A unique feature of this clinical trial is the use of the Cooperative Agreement Grant as the funding mechanism. Use of this instrument was approved by the Director, NIH, and the Assistant Secretary for Health, DHHS, and represents the first Departmental authorization to utilize this new funding mechanism for a research program of this nature. The Cooperative Agreement Grant allows the greater administrative flexibility that is needed for efficient management of this study and enables the participation of program staff as a full partner with the grantees in all aspects of the trial. However, the lack of detailed guidelines for this new partnership role by staff has presented some problems, and resolution of many of these issues has required, and will continue to require, active dialogue between various components of NIH and Institute staff. Despite these problems, it is clear that use of the Cooperative Agreement Grant is the most effective funding instrument to accomplish the specific goals of a study of this type.

A major unresolved problem associated with this new initiative is the need for stability of funding. This trial addresses a critical clinical issue in diabetes which can only be resolved through a controlled clinical trial. The nature of this type of study requires both long-range planning and a long-term commitment of support by the Institute. Given the nature of the annual budget process, this may present difficulties, and the success of this undertaking is dependent on overcoming this obstacle.

Diabetes Centers Program

Background. This program provides support for both Diabetes-Endocrinology Research Centers (DERC) and Diabetes Research and Training Centers (DRTC). The primary requirement for establishment of either a DERC or a DRTC is a strong base of high-quality, ongoing biomedical research. DRTCs have, in addition to a research component, a training/information transfer component which includes training of both medical and allied health professionals, continuing education, model demonstration, and outreach activities. For both types of centers, the grant provides for core facilities (shared resources), pilot and feasibility studies (new initiatives), and limited funds for program enrichment. This funding is intended to strengthen existing programs, increase productivity, and foster the generation of new ideas and innovative research approaches.

While the emphasis in the DERC is focused primarily on biomedical research, the fundamental objectives of the DRTC are broader, and include the creation of an environment which fosters and integrates the efforts of basic and clinical scientists, physicians, allied health personnel, and educators joined together in a cooperative, multidisciplinary approach to the many research and health care problems associated with diabetes and its complications. Therefore, the DRTC program represents not only a concerted effort to achieve a better understanding of diabetes through biomedical research, but also to improve the delivery of health care to the diabetic patient through training and continuing education programs. Although each center is expected to develop its own independent program in accordance with local needs, interests, and resources, each is also expected to be responsive to national needs, and must be willing to work cooperatively with the NIADDK and, in the case of DRTCs, with other federally-supported diabetes programs.

To provide a forum for interaction among the centers, the NIADDK sponsors an annual center meeting in June of each year, in conjunction with the national meeting of the American Diabetes Association. Participants include investigators from the centers, the NIADDK staff, and representatives from other Federal agencies with diabetes programs which interface with center activities. These meetings often lead to new ideas or avenues of approach for cooperation and collaboration which can be explored subsequently by smaller groups throughout the following year.

Presently Funded Centers. During the past year, the program provided support for 3 DERCs and 8 DRTCs as follows: Diabetes Endocrinology Research Centers--University of Pennsylvania (Philadelphia), University of Washington (Seattle), University of Iowa City (Iowa City); Diabetes Research and Training Centers--Albert Einstein College of Medicine (Bronx), Indiana University (Indianapolis), University of Michigan (Ann Arbor), Washington University* (St. Louis), University of Chicago* (Chicago), Joslin Diabetes Foundation, Inc. (Boston), Vanderbilt University* (Nashville), University of Virginia* (Charlottesville).

A mechanism of support has also been developed which allows DERCs the opportunity to become DRTCs. A DERC may apply for a modest exploratory grant to develop the resources necessary for initiation of DRTC training and information transfer activities.

Research Activities at the Centers. The vast majority of funding of research at the centers comes from NIH R01 and P01 (or equivalent) grants to center investigators. However, the concept of shared biomedical resources provided by the center funding allows for enhanced productivity and increased multidisciplinary efforts. The following examples may serve to illustrate this point.

At the Washington University, St. Louis, DRTC a variety of studies related to transplantation of pancreatic islet cells are in progress in several laboratories from various sections of the medical school representing different disciplines. During the past several years, center participants have developed and refined techniques to permit successful islet transplants between animals of different genetic strains. Work is being continued on these studies which have potentially important implications for the future of islet cell transplantation in human diabetes. Three separate DRTC core facilities, Radioimmunoassay, Morphology, and Tissue Culture, have contributed significantly to this work.

Another example of the multidisciplinary approach comes from the University of Chicago where center participants from several basic and clinical science laboratories have been investigating the structure and function of the pancreatic hormones, insulin and glucagon. These investigators have continued their studies on an abnormal insulin from a patient with diabetes caused by a mutation in the insulin gene. During the past year, the structure and other characteristics of this abnormal insulin have been elucidated. This variant insulin not only is unable to act normally to regulate the body's metabolism of glucose, but also interferes with the action of normal insulin. This research, resulting from the collaborative efforts of several researchers, represents the discovery of a new cause of diabetes in man, and contributes significantly to our understanding of diabetes. Three core resources supported by the DRTC were utilized in this study: Islet and Cell Isolation and Culture, Peptide Purification and Structural Analysis, and Radioimmunoassay.

Pilot and feasibility studies are another aspect of the research component of centers. The center awards provide modest support for three years or less to allow researchers to explore the feasibility of a concept and collect sufficient data to apply for independent funding. This mechanism provides a unique opportunity not only for testing new ideas, but also for young investigators and established investigators in other disciplines to apply their expertise in the areas of diabetes.

---

*Previously supported as a DERC.

For example, a new pilot and feasibility study at the University of Pennsylvania DERC brings together an established investigator in diabetes research with an expert in computer simulation to test the feasibility of applying these techniques to construct a model of gylcolysis in pancreatic beta cells. This model based on information concerning the properties of individual enzymes in the gylcolytic pathway determined *in vitro* will be compared with the observed behavior of islet beta-cells. The utilization of this tool may provide valuable insight into adaptive control mechanisms in finite structures. The core resources will be used in the biomedical research effort; in addition, this represents the initiation of an interdisciplinary effort which would not have otherwise been possible.

At the Albert Einstein/Montefiore DRTC, a young investigator is studying the products and mechanism of glucose-6-phosphate and glucose with hemoglobin and red cell membrane components utilizing nuclear magnetic resonance and column chromatography. The observations from this study should indicate whether such studies will extend our understanding of the mechanism of specific tissue damage in diabetes.

The centers have also been instrumental in undertaking studies on the psychosocial problems of the diabetic patient, a heretofore neglected area of research. The University of Virginia DRTC has two pilot and feasibility studies investigating methods for improving patient compliance. The Vanderbilt and Joslin DRTCs are also supporting work in this area.

Activities of the Training and Information Transfer Component at the DRTC. Prior to establishment of the DRTCs, each of the center institutions provided training and educational experiences based primarily on traditional didactic methods. With the establishment of the DRTC program, centers have been able to enlist the aid and expertise of specialists with knowledge and experience in the most contemporary methods of health education and evaluation. This has enabled centers to develop and test innovative and more effective ways of approaching training and education of health professionals concerned with the diabetic patient.

Although biomedical research is a primary focus at the DRTCs, the long range goals of these centers include the transfer of this knowledge base from the research laboratory to the wide spectrum of health professionals engaged in the care, education, and management of the diabetic patient. This includes development of a national resource of programs which provide excellent and effective training of health professionals on the most current methods of diagnosis, management, and treatment of diabetes and its complications. Developed and tested training and information transfer activities are then disseminated to a broader spectrum of health professionals. Thus, the product of this center component is development of "exportable" programs for training health professionals.

Recently, the University of Chicago completed the development and evaluation of manuals for continuing education programs for both nurses and dieticians. Copies have been deposited with the National Diabetes Information Clearinghouse in order to increase the knowledge of the availability of these manuals. The manuals themselves can be obtained directly from the University of Chicago DRTC. Additionally, one DRTC (University of Michigan) has compiled and published a catalog entitled "Audiovisual Resources for Diabetes Education," and will update this resource semi-annually.

Within the Training and Information Transfer Component at the Einstein-Montefiore DRTC, the formation, preparation, and implementation of a multidisciplinary Diabetes Education Team represents a significant accomplishment. This team provides teaching, evaluation, and consultation services to both its parent medical center and local agencies. Comprised of an internist, social worker, nutritionist, nurse, and podiatrist, the Diabetes Education Team has undertaken a variety of activities that make it the focal point of training and information transfer at this center. This team, by giving equal importance to the psychosocial and biomedical aspects of diabetes, serves as a "model" not only for the education of the health professional, but also for the diabetic patient. Recently, training programs for teams at other medical facilities have been initiated.

The training and information transfer components of DRTCs are also benefitting medical education in their host institutions. For example, the Vanderbilt DRTC offers fourth year medical students a clinical elective in diabetes which differs substantially from the traditional curriculum. Students observe and work in the DRTC Model Demonstration Unit two half days a week for four weeks. Student time is divided between lectures, participation in the care of both adult and pediatric patients, and case discussions with the health care team. Medical students attend lectures given by different health professionals: a nurse, dietitian, a clinical psychologist, and physicians. Nursing, dietary, psychology, and education students also attend the medical student lecture series, enhancing the team approach to the care of the diabetic patient.

The transfer of useful approaches to diabetic patient care is enhanced by developing relationships between the centers and other Federal agencies which support diabetes-related programs. DRTCs and nearby CDC Diabetes Control Projects have been exploring possible collaborative efforts and ways to exchange information. The centers also cooperate with the Health Services Administration's Indian Health Service (IHS) Diabetes Program. The IHS Diabetes Program is continuing to determine the feasibility of providing education and training on diabetes to Indian Health Service personnel at five selected locations. Arrangements among the Indian Health Service, the NIADDK, and the DRTCs have been made which will allow the Director of this program to utilize some center personnel in conducting these training programs. This experience is valuable to the DRTCs in evaluating the effectiveness of certain training programs in populations and cultures otherwise not available to the centers. This collaboration also benefits the IHS program by providing needed medical expertise in diabetes for its training program.

Thus, the DRTCs offer a unique resource for interaction between traditional biomedical research and health care delivery activities and are clearly excellent places for the development of programs which reflect the latest research advances applied to training and continuing education for health professionals. However, the widespread application of these programs is clearly outside the mandate of the NIH. Therefore, funding for the application of such programs must come from the other Federal, State, and/or private agencies with interest or responsibilities in health care delivery. Realization of the full potential of the DRTCs in the future will depend on the full cooperation and support of other Federal agencies through their health care delivery programs and activities.

National Diabetes Data Group

The Data Group serves to foster, coordinate, and integrate national resources for the collection and analysis of data on diabetes in order to provide a sound basis for the development of scientific priorities and the planning of public health programs. The Data Group also continues to provide the federal focus to which both the lay and scientific communities turn for accurate, reliable statistics on diabetes.

The National Diabetes Data Group consists of a nationwide group of expert advisors including epidemiologists, individuals with expertise in the biomedical, nutritional, and socioeconomic aspects of diabetes, and representatives of federal agencies (the National Institutes of Health, the Center for Disease Control, the National Center for Health Statistics, and the National Diabetes Advisory Board) and the private sector (the American Diabetes Association and the Juvenile Diabetes Foundation).

The Data Group continues to work in close collaboration with the National Center for Health Statistics in assessing the national data on diabetes collected by that agency. With the Division of Health Examination Statistics, the Data Group is analyzing oral glucose tolerance tests on a statistical sample of the U.S. population to develop norms and standards for plasma glucose levels and to determine the prevalence of undiagnosed diabetes and impaired glucose tolerance. This Division will also be conducting a study of Hispanic-Americans, for which the Data Group developed the medical history and household questionnaire, and the clinical and lab tests to be used. An analysis of the changing prevalence and incidence of diabetes over the last forty years was completed in collaboration with the Division of Health Interview Statistics.

The Data Group is also collaborating with the National Diabetes Information Clearinghouse in analyses of data collected through a special diabetes questionaire which was a supplement to the 1976 Health Interview Survey. These analyses include quantitation of the rate of diabetes among parents and siblings of diabetics, borderline diabetics and nondiabetics; the prevalence of cardiovascular risk factors among diabetics, including hypertension, obesity and smoking; the prevalence of macrovascular and microvascular disease; history of large birthweight babies; and the rate of diabetes among age/income/education groups.

The Data Group has collaborated in the development of several conferences, including "The Third Symposium on Diabetes in Asia and Oceania" (February 1981) and "Advance in the Epidemiology of Diabetes" (to be held May 1982 and cosponsored with WHO and INSERM). The summary of the conference on "ChlorpropamideAlcohol Flushing and Diabetes" sponsored by the Data Group in June 1980, was published in the journal of Diabetologia.

The Director of the Data Group presented an assessment of the diabetes data from HANES II to the annual conference of the State Diabetes Control Programs sponsored by the CDC, for use by the States in development of the epidemiologic components of their programs. The Director also collaborated with the National Cancer Institute in analyzing geographic/race/sex variations in diabetes mortality, which was presented at the June 1981 meeting of the American Diabetes Association.

National Diabetes Information Clearinghouse

NIADDK established the National Diabetes Information Clearinghous (NDIC) in September 1978 to serve as the central resource for the collection and dissemination of information about educational and scientific materials, programs, and other resources relevant to diabetes. To this end, the Clearinghouse strives to increase the availability of information for health care professionals, for patients, and for their families; increase community awareness and understanding of diabetes as a major health problem; and establish a viable system for speedy communications about new developments, techniques, and programs in diabetes among all sections of the diabetes community.

Now in its third year of operation, the NDIC has located, abstracted, and entered on a national data base over 2,700 items. These materials range from pamphlets to books, from tape programs to films. Annotated bibliographies on a variety of diabetes-related subjects continue to be in great demand from health care providers throughout the world. These bibliographies have allowed clinicians, educators, and others to locate the most appropriate materials for their needs. NDIC staff have refined a materials evaluation guide and are actively encouraging producers and users to evaluate these materials to measure their potential value. Health professionals are sending format evaluation to NDIC for inclusion in future bibliographies.

Two major publications were compiled and edited by NDIC staff in the past year: Behavioral and Psychosocial Issues in Diabetes: Proceedings of a National Conference and State and Federal Assistance: Resource Directory for People with Diabetes. Seven (7) new or revised annotated bibliographies were also published and distributed.

The NDIC continues to work closely with voluntary and professional diabetes organizations as well as with other Federal agencies. In particular, the NDIC is providing support to evaluate special educational materials developed for American Indian groups in cooperation with the Indian Health Service, HSA. A major educational conference scheduled for September is being coordinated by NDIC and representatives from the 20 CDC supported Diabetes Project states have been invited to participate. Additionally, NDIC will work with a number of Federal agencies to test and distribute a Diabetes Primer, written at the urging of and under the guidance of the National Diabetes Advisory Board.

As knowledge of the NDIC has spread, requests for information have increased from a modest beginning of 20-30 requests per month to almost 1,000 each month.

In order to assess the effectiveness of and need for the bimonthly newsletter Diabetes Dateline as directed by OMB, the readership was asked to return a card if they wished to remain on the mailing list. Over two thirds of the 3,000 readers wished to continue receiving the newsletter. Additionally, these respondents indicated that about 5 other people read each issue, giving us a readership of about 10,000 for an extremely modest cost.

The program has applied for and been granted evaluation set-aside money for an evaluability assessment of the NDIC. The results of this assessment will be available early in the next fiscal year.

ENDOCRINOLOGY, METABOLIC DISEASES, AND RESOURCES PROGRAMS BRANCH

This Office coordinates the Endocrinology, Metabolic Diseases, Research Training and Development, and Resource Development Programs of the Cluster. In addition, the office serves as the focus for planning and implementing special initiatives and for administering the NIADDK cystic fibrosis-related activities.

Endocrinology Research Program

A major emphasis of the Endocrinology research program is to support studies of how hormones act at the cellular and subcellular level in the organism. This program supports a number of studies that attempt to determine the relationship of hormonal structure to function and the role played by cellular and subcellular membranes in hormone action. Other areas of emphasis include studies on secretion mechanisms of hormones, hormonal precursor molecules, and the synthetic processes used by the cell to produce hormones. In addition to these basic studies, the program also supports a number of clinically related studies on the pituitary, thyroid, parathyroid, and adrenal gland hormones. Studies on more recently discovered hormones, e.g., peptides from the hypothalamus such as endorphins and enkephalins and on hormone-like substances, such as prostaglandins, are also supported.

The following are some highlights of research supported by the Endocrinology Research Program in 1980.

Growth Hormone. We have reported in previous years the progress in production of rat and human growth hormones in bacteria by means of recombinant DNA techniques. Similar techniques have now been used to localize the genes for human growth hormone and chorionic somatomammotropin (also called placental lactogen) on chromosome 17. Localization was accomplished by using complimentary DNA (cDNA) fragments containing the codes for their genes. Mouse-Human cell hybrids were then developed which contained various combinations of human chromosomes mixed with the full complement of mouse chromosomes. Studies showed that the cDNA probe combined only with those cells containing the human chromosome 17, thus localizing the genes to that chromosome. This finding may have importance to human medicine in the future for treatment of patients deficient in these hormones.

Other studies of human growth hormone indicate that the hormone may actually be a complex of proteins which exhibit different activities, thus accounting for the variety of hormone action found with growth hormone. One variant of hGH, known as the 20,000-dalton variant, has been shown to have 15 fewer amino acids than the normal hGH molecule. The loss of these 15 amino acids abolishes the insulin-like properties of normal hGH, as well as its hypolytic activity. It does not, however, affect the growth promoting activity. Using recombinant techniques, investigators hope to show which portion of the GH molecule exhibits the growth promoting, diabetogenic, insulin-like and lactogenic activities of the molecule. Hybridization with GH from other species may show the difference between species of this hormone, and explain why the hormone from other animals does not work in man.

Thyroid Studies. One investigator is studying the role of the adrenergic portion of the autonomic nervous system in the regulation of thyroid gland function. Using the cat as a model, he found that thyroid cells contain both alpha and beta adrenergic receptors and that adrenergic nerve endings are in close proximity to the follicular cells. He also found that epinephrine and norepinephrine stimulates the alpha-adrenergic receptors in the cat thyroid, resulting in a 40-50% reduction in the TSH stimulation of cyclic AMP accumulation. The mechanism for this inhibitory effect is unclear; however, the hypothesis is that epinephrine causes increased uptake of tissue calcium which in turn activates a phospholipase. The phospholipase enzyme then is thought to release arachidonic acid, a metabolic product of which is believed to inhibit TSH stimulated adenylate cyclase. This same investigator has also observed that insulin in low concentrations suppresses TSH stimulated cyclic AMP formation, apparently by a different mechanism. Studies such as these show the complex interactions of hormones with the nervous system in the body.

Calcitonin. Calcitonin together with parathyroid hormone helps control bone growth and resorption. Calcitonin biosynthesis has been studied using calcitonin producing tumors of rats. Various forms of calcitonin have been identified, with molecular weights of 25,000, 15,000 and 9,000. By using recombinant DNA techniques and various methods of analysis, researchers have determined that the initial synthesis of calcitonin is in a "pre-pro" form of relatively high molecular weight. This form is biologically inactive. Enzymatic splitting to an intermediate form produces a "pro calcitonin", which is then split to the final biologically active form of about 9000 molecular weight. Thus, calcitonin appears to be formed in a manner similar to other hormones from a large precursor molecule.

ACTH. ACTH is one of the pituitary hormones whose main effect is stimulation of the adrenal cortex. One group of investigators has shown that ACTH is also produced in the amygdala, a portion of the brain. In addition, they have studied the effect of ACTH on mental function. Work in rats has suggested that ACTH has a direct effect on higher brain function. To see if there is an effect in humans, these investigators studied patients with ACTH deficiency due to tumors of the pituitary. The patients were interviewed and given psychological tests that emphasized memory and recall. They were then treated in a doubleblind study with ACTH. In all cases the patients with ACTH and other pituitary hormone deficiency had significant defects in memory and concept formation. However, ACTH in the doses given over a relatively short period of time did not restore function to normal. The conclusion is that:

1. The pituitary does secrete a hormone or hormones influencing memory and concept function, but it is not ACTH, or;

2. ACTH has to be given for a much longer period of time to be effective or;

3. ACTH deficiency, once developed, may produce irreversible brain changes. Then studies are important especially to the aging process, since the brain deficiences studied were very similar to those found in senility.

Growth Factors. There are several growth factors known which affect growth either directly or in conjunction with other hormones. Somatomedin (or insulin-like growth factor) appears to work in conjunction with growth hormone. For example, one clinical study of short children showed that the growth hormone level was normal, as determined by radioimmunoassay, but somatomedin levels were low. When GH was administered, serum somatomedin increased and the children began to grow. Studies showed that the GH was probably abnormal, even though the amount appeared to be within the normal limits. It also shows that GH appears to work through somatomedin.

Somatomedin appears also to serve as a feedback control mechanism to GH production. As somatomedin levels increase in the blood, the amount of somatostatin increases. Somatostatin is a known inhibitor of growth hormone, thus the level of somatomedin regulates the level of growth hormone.

Hormone Distribution Program. This program distributes pituitary and other hormones and various antisera to cinical and basic science investigators in this and other countries. The distribution program has been very helpful to many investigators since its inception. During the past year, preparations have begun for recompeting the contracts through which the distribution program is operated. An ad hoc advisory group of leading investigators in endocrinology was convened to review the current activities of the program and to recommend any changes in its scope and/or mode of operation. Principal recommendations included:

1. The addition of several peptides to the offerings of the program including somatomedin-C and beta-endorphin.

2. The collection and distribution of parathyroid tissue to qualified investigators.

3. The use of an independent organization to conduct assays of chemical purity and biological activity on the products to be distributed.

Metabolic Diseases Research Program

The primary objectives of this program are to support investigator initiated studies of basic metabolic processes underlying all diseases within the purview of the Institute and basic and clinical studies of various inborn metabolic diseases. Major foci of basic research investigations include: membrane structure, biological transport and enzymatic mechanisms, and their roles in the regulation and control of normal as well as inborn or acquired abnormal metabolic processes. Additional areas of emphasis reach into the diagnosis, etiology, pathogenesis and treatment of a large number of inborn metabolic diseases. Some highlights of research accomplishments during FY 81 are summarized below.

Structure and Function of Proteins, Peptides and Enzymes. Biological effects of drugs often depend on the way in which they interact with proteins at the site of their action. The protein, functioning as a receptor for the drug, binds it at an active site which has a specific location, geometry and chemical composition. Our ability to mathematically model binding sites of various proteins and enzymes as well as to predict drug-protein interactions is of extreme importance for understanding drug action and to design better drugs. A research team, supported by the program, is working on development of a novel distance geometry approach for study of receptor-drug binding utilizing computer assisted analysis of binding site shape and energetics. The newly developed methodology was applied to a series of analogs of the hormone thyroxine, for which knowledge of receptor-hormone complex structure was available from X-ray crystallographic measurements. The developed methodology not only duplicated the already known structures but was able to predict binding free energies for three analogues intentionally excluded from the data set. If further validated on other systems, the method could be a major contribution and lead to improvement in the therapeutic effects of many drugs.

Glutathione is a tripeptide with an as yet undefined role in the extracellular formation of the amino acid cystine. This role is studied with the aid of the drug buthionine sulfoximine, an inhibitor of the enzyme gamma-glutamylcysteine synthetase. While studying the properties of the inhibitor, a group of researchers found that the protozoan Trypanosoma b. brucei, the causative agent of Nagana, or animal Trypanosomiasis, is killed when exposed to buthionine sulfoximine. This drug acts on the parasites, in infected mice, by depleting their glutathione content. Glutathione is necessary for the disposal of hydrogen peroxide produced intracellularly by the parasites which lack the enzymes to detoxify peroxides. Due to the depleted glutathione, the accumulating peroxide kills the parasites. Since mammalian cells contain peroxide detoxifying enzymes, buthionine sulfoximine is apparently non-toxic to the host. These findings suggest a new viable alternative to the treatment of Trypanosomiasis, with possible applicability to treatment of African sleeping sickness or in South America, Chagas disease. These studies are an excellent demonstration of how discoveries in seemingly unrelated scientific research areas may have direct pay off in the treatment of human diseases.

Studies on the roles of proteins in transport through membranes; their location on the outer or inner surface of membranes and their three dimensional structural arrangement in membranes are major targets of today's research efforts. A membrane impermeant, cleavable protein crosslinking reagent, capable of facilitating such studies, was synthesized recently. This reagent, called DIDIT, specifically crossreacts with amine groups of a known transmembrane protein at the outside face of intact human erythrocyte membrane and it is removable from the membrane under mild conditions. This first successful demonstration of the crosslinking of a membrane protein with an outside crosslinker only at one face of a biological membrane should significantly facilitate research in all aspects of membrane structure - function and foward our understanding of vital processes at the molecular level.

Regulation of Metabolic Pathways. Phosphofructokinase (PFK) is a key enzyme in the glycolytic pathway and is responsible for the irreversible formation of fructose-1,6-diphosphate (FDP), itself a key intermediate of the same pathway. Until recently, PFK was thought to be regulated through inhibition by high concentrations of adenosinetriphosphate (ATP), or stimulated by adenosine diphosphate and monophosphate. A research team has recently isolated a highly labile compound, fructose-2,6-biphosphate (FBP) from rat liver perfused with high concentration of glucose. FBP has the ability to stimulate the activity of PFK at extremely low concentrations by increasing the affinity of the enzyme for its substrate, fructose-6-phosphate, and by relieving the inhibition by ATP. It was found that the concentration of FBP is increased in the presence of glucose; decreased by glucagon and affected by the hormone vasopressin and by lactate. The discovery of this compound, which appears to be a potential new regulator of an important step of glycolysis, could have far reaching implications in advancing our understanding of carbohydrate metabolism and its defects in diabetes mellitus.

Inborn Metabolic Diseases. Mucopolysaccharidoses (MPS) VI, or the Maroteaux-Lamy syndrome, due to deficiency in the enzyme arylsulfatase-B, can be a severe disease characterized by stunted growth, joint stiffness, corneal clouding, severe hip deformity, etc. A group of researchers has recently identified a feline model for this disorder and isolated the enzyme arylsulfatase-B from both normal and afflicted cats. An understanding of differences between normal and mutant enzymes in cats and their comparison with arylsulfatase-B isolated from normal and afflicted humans opens the way to initiation of enzyme replacement studies in the feline model and to understanding the genetic mutation in feline and human MPS VI.

Development of the gas chromatograph mass spectrometer (GC-MS) has contributed to our ability to detect various diseases through simple derivatization of urinary metabolites. The major drawback of this methodology is the high cost of such instruments and thus their relatively sparse availability. Gas chromatographic identification of 155 derivatized (trimethylsilylated) important organic acid metabolites from urine, as recently reported by a research team, allows for relatively inexpensive diagnosis of various inborn organic acidurias. The availability of this convenient diagnostic method allows initiation of screening programs for organic acidurias in hospitals not equipped with GC/MS.

Cystic Fibrosis (CF). Several investigators have continued their efforts to develop ways of detecting carriers of the CF gene (heterozygotes) and of performing prenatal diagnosis of the disease. Although a number of approaches are being explored in each case, none has been developed and validated to an extent that would allow widespread use at this time. A recent report indicated that fibroblasts from both CF patients and carriers tended to accumulate less sodium than normal fibroblasts after exposure to ouabain; however, initial attempts to replicate the effect have been problemmatic. Work has continued in the development of a prenatal test for CF using amniocentesis. The test is based on studies showing lower than normal levels of protease activity in the amniotic fluid of CF pregnancies. A number of high risk (for CF) pregnancies are currently being followed to term. To date, all infants subsequently found to be normal were predicted to be normal by the test; however, several infants subsequently found to have CF had test results in the normal range. Efforts to perfect this test are continuing.

The program also supports a number of basic research projects examining the secretory process in general. The structure and function of sweat and mucous producing glands are of major interest because of their aberrant functioning in CF patients.

Previous annual reports have described efforts to increase investigator awareness of the needs and opportunities in CF research, and the numbers of applications received by the program increased markedly in 1979 and 1980. This trend halted in 1981, however, with the same number of applications being received as in 1980.

Training and Career Development Program

The Training and Career Development Program continues to pursue its mission of attempting to assure an adequate supply of well-trained investigators in areas of the Cluster's responsibility; however, shortages of funds in both the training and career development categories have sharply curtailed the new and competing awards that could be made in 1981. The number of trainees supported by new and competing awards (both institutional and individual) in 1981 was less than one-third of the number supported in 1980. Virtually no new career development awards were made in 1981. If these trends continue, a major effort must be made to develop priorities among the various types of awards offered within the program to assure that the limited resources available are allocated in the most effective way.

Research Resources

The Cluster continues to support the development and distribution of various research resources to qualified investigators; however, budgetary limitations prevented the expansion of such support in 1981. The activities of the National Hormone Distribution Program (formerly the National Pituitary Agency) were reviewed by an ad hoc advisory committee and its recommendations are summarized elsewhere in this report. Several insulins and insulin analogues are available through the program.

ARTHRITIS, MUSCULOSKELETAL AND SKIN DISEASES PROGRAM

ANNUAL REPORT - 1981

ARTHRITIS RESEARCH AND TRAINING PROGRAMS

Investigator-initiated research in the Arthritis Program can be categorized by four major areas of emphasis. The first includes general approaches to the rheumatic diseases concerned with etiology, metabolism, inflammation, humoral and cellular disturbances, autoimmunity and specific antibodies. The second is research differentiated to categorical diseases such as rheumatoid arthritis, sytemic lupus erythematosus, degenerative joint disease, and heritable disorders of connective tissue. The third is research which is not disease specific, related to articular cartilage, collagen, mucopolysaccharides, basement membrane, and synovial tissue and fluids. Fourth, and lastly, are studies associated with structure, function, and metabolism of skeletal muscle, together with the physical and biochemical events associated with muscle excitation and contraction.

Highlights and summaries of research objectives and results from individual research projects along with current proposed researches are reported within areas of major interest that comprise the program.

Collagen and Connective Tissue

Support for a four-day international conference to help define important problems and future directions for research on the biochemistry and molecular genetics of collagen was provided to promote the application of the technology of molecular genetics to the connective tissue field. The conference consisted primarily of three concurrent Discussion Group Sessions and was patterned after the Dahlem Conferences in West Germany - of proven success - which emphasize small discussion groups followed by plenary sessions. The three discussion groups were charged with covering the following topics: Group I: Structure of Collagens, Collagen Genes and Procollagen; Group II: Regulation of Collagen Biosynthesis and Degradation; Group III: Genetic and Acquired Diseases Involving Collagen.

The conference was timely, addressing important areas of interest and recognized the importance of recent advances in molecular genetics, including the availability of techniques of recombinant DNA which make the genes for collagen directly accessible for use in sophisticated experiments and as potent tools in the diagnosis of clinical disorders. The rationale and specific aims, as well as the topics covered, were focused on the structure of collagen, procollagen and the genetic expression of collagenous proteins in normal and disease states. The plan to produce a monograph summarizing the conference will provide other workers with an excellent description of the field. The monograph will include materials written prior to the conference as well as summaries of the discussions during the conference. A major hurdle has been approached in the connective tissue field with the application of some of the new technologies of molecular biology to the study of the regulation of connective tissue component biosynthesis. Bringing together experts in both the connective tissue field and the molecular biology field will provide proper means to encourage workers in the connective tissue field to move in these directions.

Recent research has produced evidence of a new collagen type, which has been called AB or type V. This collagen is considered distinct from other collagen types by a number of criteria, including solubility, gel electrophoresis, amino acid composition and cyanogen bromide peptide pattern. Studies now in progress should complete the characterization of type V collagen, including the question of whether two chains exist in the same molecule or whether separate proteins of different composition exist. Isolation and characterization of the cyanogen bromide peptides for the two chains is being accomplished as well as the effort to determine whether the chains exist in one or two different molecules. Biochemical analyses and immunofluorescence techniques are being employed to determine the distribution of this protein together with studies of its role in normal and pathophysiology and disease states in which this protein may be involved, including hereditary disorders such as the Marfan syndrome.

Etiology of Rheumatoid Arthritis and Systemic Lupus Erythematosus

The principal thrust of an important project is to identify microbial agents suspected to be etiological factors in rheumatoid arthritis and systemic lupus erythematosus. To this end, attempts are being made to identify and characterize microbial DNA in the plasma and tissues of patients. The techniques are based on nucleic acid hybridization, protein renaturation and nucleic acid probes. Progress thus far has not produced conclusive evidence of microbial DNA in the plasma of patients but the studies have the potential of revealing valuable information and the medical significance of this project is extremely high.

Circumstantial evidence exists that a permissive background and a persistent but inapparent infection underlies these collagen disorders. Two approaches are being taken. In one, DNA will be isolated from the circulation to determine if it is of foreign origin. In earlier studies, DNA isolated from eight subjects with lupus did not renature with known DNA suggesting that a base sequence difference might be present. These studies are being continued to determine if the explanation is in the methods or if there are true differences between base sequences and those of human nuclear DNA. If the latter is true, it will be characterized to determine its origin. The second approach requires preparation of DNA probes of high specific activity in the attempt to detect DNA from microorganisms in tissue. Important new modifications in the traditional methods for DNA reassociation will be used. One of these techniques, the Phenal Emulsion Reassociation Technique (PERT), allows renaturation to take place at room temperature and at substantially enhanced rates while permitting the screening of DNA in small samples of tissue or blood in a large number of samples.

Efforts are also underway to make a large-scale search for genera that may be of etiologic significance. Broadly specific DNA probes will be prepared from those genera of organisms that appear most likely to be of etiologic significance. Probe sensitivity will be enhanced by using a variety of restriction endonucleases to select out the desired DNA.

Systemic Lupus Erythematosus

Abundant evidence has been given that the deposition of immune complexes is the primary event which leads to tissue injury in blood vessels, glomeruli and the skin of patients with systemic lupus erythematosus. Investigations of the pathogenetic roles of immune complexes in this disease continue to further define the immunochemical properties of circulating antibodies and antigen-antibody complexes, using enzyme immunoassays and correlating these with the clinical and histologic findings in patients with lupus. Attempts are being made to isolate complexes found *in vivo* or formed *in vitro* and determine their physical, immunochemical and biologic properties. Comparisons are being made between the characteristics of immune complexes associated with injury when deposited in tissue and those whose deposition is not associated with tissue damage. Overall the main thrust of these investigations is to identify the peculiar characteristics of the variety of immune complexes found in lupus sera which relate to clinical manifestations of the disease. Much pioneering work has been done within this project. The emphasis has shifted from studies of the nature and source of the nucleic acid antigens found in the sera of patients to the characterization of the immunochemical specificity, physical and biologic properties of the antibodies found in such sera. One of the major features now involves the use of enzymatic immunoassays for the detection of antibodies specific for as many as twenty different antigens.

Rheumatoid Arthritis

It is important to understand the proliferative and destructive events that occur in rheumatoid arthritis in which the breakdown of collagen in cartilage, bone, and other tissues is prominent. Synovial collagenase is central to the destruction of collagen in arthritis. It is important to understand the physiology and biochemistry that regulate the production of this enzyme, the mechanisms of intracellular transport, the storage and secretion of the protein and the fate of latent collagenase in extracellular tissues after release from cells. A number of interesting model systems, including synovial cells stimulated to produce collagenase by phorbol myristate acetate and the inhibition of collagenase production in such stimulated cells by the compound retinoic acid,are being used to examine these factors. The stimulation or inhibition of collagenase production by these compounds is being related to changes in phospholipid and prostaglandin metabolism. Collagenases produced by rabbit synovial fibroblasts and rheumatoid synovial cells are being purified to produce monospecific antibodies for studies of intracellular localization of the enzymes and specific inhibitors of collagenase from tissue are also being purified for study in their own right. Finally, because of the difficulty in obtaining large amounts of rheumatoid synovial cells for this work permanent cloned lines from both normal synovial and rheumatoid synovial x-irradiated cells will be established and characterized with respect to growth, collagenase and proteinase production, inhibitor synthesis and prostaglandin metabolism.

Current methods for the treatment of rheumatoid arthritis are far short of being satisfactory. Corticosteroids appear to inhibit collagenase production, but they affect many other cellular functions and some of these effects are adverse. More selective inhibition of collagenase production is therefore needed and work is in progress to examine the effects of retinoic acid first *in vitro* and then in experimental models of arthritis. Clinical trials may be started if the results are promising. A fair knowledge of the basic components in the system, mainly the cellular handling of collagenases and inhibitors, cellular phospholipid and prostaglandin biosynthesis and the metabolic interrelationships among these several aspects of cellular function must be available to design a maximally effective mode of therapy.

Crystal-Induced Arthropathy

The influx of polymorphonuclear leukocytes plays a crucial role in initiating the inflammation associated with crystal-induced arthropathy. It has been demonstrated that following phagocytosis of urate or calcium pyrophosphate crystals, polymorphonuclear leukocytes release a low molecular weight glycoprotein which is chemotactic. The relevance of this factor to crystal-induced arthropathy was suggested by two findings. First, injection of purified factor into the joints of rabbits resulted in the rapid onset of inflammation which was indistinguishable from that seen in crystal-induced disease. Second, polymorphonuclear leukocytes displayed specific receptors for the factor.

Studies on this factor will include comparative quantification in the synovial fluid of individuals with crystal versus noncrystal-induced arthropathy; delineation of other materials such as antigen-antibody complexes which can induce synthesis; and whether the factor can inhibit phagocytosis and thus play a role in the spontaneous remission of inflammation that occurs after polymorphonuclear leukocyte ingestion of crystals. Another project will look at the amino acid sequence of this factor. The last project will explore metabolic and biochemical events which occur subsequent to interactions between this factor and polymorphonuclear leukocytes; this will include changes induced by the factor in membrane potentials, ion fluxes, and cyclic nucleotides.

Immune Disturbances in Rheumatic Diseases

A research group at the University of New Mexico has been able to prepare two antisera capable of detecting antigens specific for lupus by using, as immunogens, lupus lymphocytes coated with normal anti-lymphocyte antibody. Attempts are being made to extend the immunochemical studies of the antigens reacting with antisera prepared against mononuclear cells from patients with active systemic lupus erythematosus. After extensive adsorption and pepsin digestion, absorbed anti-SLE reagents will be utilized to further characterize antigens which react with these antisera and are present on peripheral blood lymphocytes as well as polymorphonuclear leukocytes. Another portion of the study will focus on attempts to learn more about idiotypic antigens associated with rheumatoid factors in patients with rheumatoid arthritis. Analysis of cross-reacting rheumatoid factors which react not only with a specific fragment of the immunoglobulin G moiety but also with nuclear antigens will be extended and attempts will be made to utilize antirheumatoid factor idiotypic antibody to identify rheumatoid factor idiotypes or materials cross reacting with the latter in body fluid, urine, and synovial tissue from patients with rheumatoid arthritis. A preparation of reagents capable of identifying T cell subclasses in peripheral blood and tissues will be extended. These antisera will be prepared against isolated specific T cells obtained from human umbilical cord bloods after extensive absorption with T cells of different specificity, human thymocyte suspensions, and a number of other cell types. Similar approaches for other T cell subsets will be initiated. Finally, the relationship between C-reactive protein binding lymphocytes and cells capable of reacting with streptococcal antigens in acute rheumatic fever will be investigated to explore whether this protein is capable of modulating a potentially harmful immune response in this circumstance.

The objectives of another research group are to study the relationship of immunoglobulin G and M-rheumatoid factor to the systemic complications of rheumatoid arthritis and to examine factors that regulate the synthesis *in vitro* of autoantibodies. They will investigate the role of immunoglobulin-rheumatoid factor complexes in the pathogenesis of rheumatoid arthritis as well as to determine immunologic factors related to their enhanced synthesis *in vitro*. Comparisons will be made between rheumatoid arthritis patients with and then without systemic complications. Soluble as well as cell-dependent factors related to controlling the *in vitro* production of rheumatoid factor by circulating synovial fluid and synovial membrane mononuclear cells from patients with rheumatoid arthritis will be studies and the specificity of the modulating factors for rheumatoid factor production will be determined. Similar investigation of modulating factors related to excessive production of rheumatoid factor and anti-DNA antibodies among the spleen cells of mice with autoimmune disease resembling human systemic lupus erythematosus will be carried out. The project will be rounded out by planned studies designed to improve the sensitivity of techniques useful for the detection of immunoglobulin G-rheumatoid factor produced *in vitro* by mononuclear cells.

Numerous circulating autoantibodies to nuclear and cytoplasmic macromolecules have been detected in patients with systemic lupus erythematosus and other rheumatic diseases. Intensive investigation of antinuclear antibodies has resulted in the discovery of additional distinct nuclear acidic protein antigens which are present predominantly or appear to be specific for a number of rheumatic disease entities. Among these are mixed connective tissue disease, Sjogrens syndrome, polymyositis, dermatomyositis and systemic lupus erythematosus. Thus, antibodies to a number of distinct nuclear antigens appear to be of diagnostic and/or prognostic value in a number of rheumatic diseases. A series of studies is in progress to continue the purification and the biochemical and immunologic characterization of a number of nuclear antigens identified as PM-1, RNP, SM and MA; to produce more sensitive assays for these materials; and to analyze the expression and localization of nuclear acid protein antigens in serum, tissue, and cells. There are plans to extend the observations made to a mouse model inbred to select for antibodies to RNP and SM and analysis of the influence of T cell function.

Muscle Structure and Function

Studies concerned with muscle contraction include projects concerned with contractile proteins, the contractile mechanism, the biology and regulation of contraction, and the mechanical control and energetics of contraction. Projects associated with the structure and function of muscle include myofibril structure, sarcoplasmic reticulum, regulatory proteins, and the structural basis and dynamics of cellular function and movement.

One study continues an effort to characterize the sarcoplasmic reticulum and surface membranes of heart and skeletal muscle both structurally and functionally in molecular terms. The overall aim is to delineate how the intracellular calcium concentration of muscle cells is regulated by these membranes. The area of research concerns the isolation and study of the various supramolecular complexes, or organelles, involved in the regulation of contraction in skeletal and heart striated muscles. The subcellular fragments or organelles involved are the surface membrane or plasmalemma, the transverse tubular system, and the internal closed membrane system known as the sarcoplasmic reticulum. The approach uses whole muscle tissue subjected to various homogenization and centrifugation procedures. Subsequent treatment yields relatively pure fractions which contain surface membrane, T-system membrane, functional fragments known as triads, and sarcoplasmic reticulum vesicles. Various techniques are being used to test purity to reconstitute various purified protein molecules involved in the transport and permeability properties of these membrane systems into lipid biplanar bilayers. The use of these bilayer systems may help in the identification of voltage dependent calcium channels in the sarcoplasmic reticulum. The studies include work with dystrophic chicken pectoralis muscle and may shed some light on the basic pathological changes which occur in the disease. The basic aim of all this work is to determine the molecular basis for the release, uptake, and storage of calcium, which is now known to be the mediator of excitation-contraction coupling in striated muscle. A notable achievement has been the finding that the sarcoplasmic reticulum contains a potassium and sodium channel which appears to be randomly dispersed throughout the reticulum structure. This channel appears to act as an independent but complementary part of the elctrogenic calcium transport system. *In vitro* assembly of the sarcoplasmic reticulum membrane resulted in random orientation of calcium-ATPase and showed that it is surrounded by an annulus of rigid lipid at low but not at physiological temperatures. A magnesium or calcium activated ATPase, the so-called "basic" ATPase has been found to be associated with the plasmalemma and T-system membranes of normal skeletal muscle. Particulate guanylate cyclase activity was found to vary with calcium concentration and its activity was increased five to tenfold in dystrophic chicken pectoralis muscle. The studies are comprehensive and imaginative and can lead to important advances in the understanding of the mechanisms involved in excitation-contraction coupling.

SKIN DISEASES RESEARCH AND TRAINING PROGRAMS

The Skin Diseases Program of the NIADDK provides research support for basic and clinical studies of the skin in normal and diseased states. Areas of emphasis include: psoriasis and disorders of keratinizing tissues; vitiligo and other disorders of pigmentation; photobiology, photo-allergy, and phototoxic reactions; metabolic studies of skin, including effects of hormones and interaction with enzymes; immunologically mediated cutaneous disorders, including atopic dermatitis, eczema, contact dermatitis, and vasculitis; bullous diseases of the skin, including pemphigus, pemphigoid, dermatitis, herpetiformis, and epidermolysis bullosa; acne and physiologic activity of the sebaceous gland; disorders of hair growth, including alopecia areata; and cutaneous manifestations of connective tissue disease disorders, including lupus erythematosus, scleroderma, and pseudoxanthoma elasticum.

The Skin Diseases Program utilizes many funding mechanisms, including regular research grants, career development awards, Clinical Investigator Awards, New Investigator Research Awards, training grants, and contracts.

Increased interest and research findings about epidermolysis bullosa brought about a workshop on disease mechanisms, held on the NIH campus in April 1981. Investigators from divergent specialties with interest in this uncommon but devastating blistering disease met to define the clinical, genetic, pathologic and nutritional parameters of importance as they pertain to the various types of epidermolysis bullosa (EB). These include: prenatal determination of predisposition to the disease; nutritional deficiencies resulting from pathological involvement of the mouth and intestinal tract; surgical removal of the mitten-like enclosures of the digits which are sometimes seen in advanced cases of EB; and dental care of EB patients. Proceedings of the workshop will be published later this year.

The Skin Diseases Program also contributed significant support to the Third International Symposium on Psoriasis held at Stanford University in July 1981. The publication of the proceedings is likely to be a major resource document for current basic and clinical information on psoriasis.

Vitiligo

The second year of a major research initiative into vitiligo has been completed. The grant, totaling approximately $2 million, was awarded to a consortium comprised of Yale University, Howard University, Bryn Mawn College, the University of Pennsylvania, the University of Massachusetts, and the Massachusetts Eye and Ear Infirmary. The overall goals of the investigators at the Yale Medical Center are to find the cause and define the mechanisms which produce vitiligo and to improve the therapy for patients. They will attempt to employ the processes which destroy normal pigment cells in vitiligo to treat patients with metastatic melanoma. The primary objective at Howard University has been to develop an accurate method to determine 8-methoxypsoralen in blood, as 8-MOP is the psoralen used to stimulate repigmentation in vitiligo. In the past year, the Howard University investigators have indeed reported a reliable and reproducible method for the determination of 8-MOP in plasma, serum, or whole blood. The results indicate that a fraction of 8-MOP is bound very strongly and appears to be released intact after denaturation and partial destruction of proteins. The significance of this work is that a simple method has been developed which is available to the medical and scientific community to establish the relationship between dosage, plasma, or blood levels of 8-MOP and the therapeutic response of patients to photochemotherapy for vitiligo and for psoriasis. The possibility of the existence of a psoralen binding protein is also suggested.

Researchers at the Massachusetts Eye and Ear Infirmary have examined the eyes of over 200 patients with vitiligo and observed ocular pigmentary abnormalities in approximately one-third of these patients. The changes frequently include discrete areas of depigmentation with associated pigment hyperplasia, clinically appearing to involve the choroid and retinal pigment epithelium. Some patients have active uveitis. Fundus appearance in a number of these patients suggests a disturbance of the retinal pigment epithelium.

Investigators at the University of Pennsylvania are examining the psychosocial effects of vitiligo. Although the depigmenting effect secondary to the destruction of pigment cells causes no physical impairment, it does leave the patient cosmetically disfigured, which is especially severe in patients with dark skin. In at least half of the patients studied, vitiligo has detrimentally impacted on their emotions, their ability to interact with others socially, and, in some instances, to obtain the occupation of their choice. Additional information obtained from these individuals will be used to improve the care of vitiligo patients by their physicians.

Investigators at the University of Massachusetts are examining inherited vitiligo in a mutant line of chicken that appears to develop spontaneous amelanosis and related thyroid and skin defects. There are eye defects as well. Initial studies indicate an increasing number of similarities between this disease in chickens and that of spontaneous human vitiligo. Further studies have been designed to reveal the basic underlying cause behind the defective melanin pigmentation in the two species.

Photobiology

The emerging discipline of photobiology has produced new data on the action spectra in light-associated diseases. Photodermatoses under study include xeroderma pigmentosum, lupus erythematosus, polymorphous light eruption, solar urticaria, actinic reticuloid, halogenated salicylanilide photosensitivity and persistent light reactors, psoralen photosensitivity, and porphyria. Normal minimal erythema dose in the UVB range was generally observed in polymorphous light eruption and lupus erythematosus. Maximum photosensitivity occurred in solar urticaria.

Blistering Diseases

Disruption at the epidermal-dermal junction occurs as the primary pathology in dermatoses, both inherited (epidermolysis bullosa) and inflammatory (lupus erythematosus and pemphigoid). Although the components of the junctions are anatomically well defined, little is known about their composion or biosynthesis except that the junction structures play a role in attaching epidermis and dermis and may function as a barrier to the movement of large molecules and cells and even neoplastic cells between the epidermis and the dermis. Thanks to the recent development of monoclonal antibody production techniques, a monoclonal antibody with specificity limited to cells in the basal and suprabasal layer of the human epidermis was produced by investigators. The investigators used immuno-electron microscopy, and found the monoclonal antibody concentrated in the sub-basal lamina area specifically associated with anchoring fibrils. Significant advances in the isolation and characterization of the structures of the epidermal-dermal junction should result from further studies.

Pharmacology

Topical glucocorticoid therapy is the mainstay of treatment of many dermatologic diseases. A significant research project attempts to demonstrate a functioning glocucorticoid hormone receptor system in human skin and to correlate this function of the system with the response of dermatoses to topical glucocorticoid therapy. Understanding of steroid and sterol sulfate metabolism in epidermis should provide important insights into normal stratum corneum shedding and aberrations of this process manifested as scaling dermatoses (e.g., psoriasis, ichthyosis).

Psoriasis

The objective of some of the recent psoriasis research projects is to determine whether the skin of patients with psoriasis is inherently disordered. There is much evidence to suggest that both the involved and uninvolved skin of patients with psoriasis appears to be equally affected, based on epidermal proliferation rates. By transplanting normal human skin and psoriatic skin to congenitally athymic mice, grantees have taken advantage of a biologic support system, devoid of humeral factors, wherein the usual structural as well as differentiation and proliferation features inherent to human epidermis persist. Using this system, grantees are comparing epidermal proliferation of the transplanted involved and uninvolved skin of psoriatic subjects with transplanted skin of normal subjects, relative to time. In addition, it will be possible to determine whether topical corticosteroids suppress the abnormal epidermal proliferative response inherent to skin after it has been transplanted.

Acne

A dramatic response of severe and treatment-resistant cases of cystic and conglobate acne occurring following the use of oral 13-cis-retinoic acid was reported last year. The mechanism proposed for this action is the marked inhibition of the synthesis of sebum, the secretory product of the sebaceous gland which plays a major role in the pathogenetic sequence leading to inflammation in acne. The original group of patients has now been followed for over three years and has maintained the clinical improvement, although some of them have required a second course of treatment. Several of the patients have continued to have suppression of sebaceous gland activity, which is supportive of the theory that the major activity of this drug is mediated through sebaceous gland inhibition. Recently a more extensive study has been conducted to compare 13-cis-retinoic acid with an aromatic derivative (Ro-10-9359) which appears to be extremely effective in controlling psoriasis. When administered in an equal dose the aromatic derivative is approximately one-third as potent as 13-cis-retinoic acid in inhibiting sebaceous gland function. This finding is of extreme importance, for it indicates that there is molecular specificity in the action of the retinoids administered orally, and that it is possible that other retinoids will be found that will be equally effective with fewer side effects.

MUSCULOSKELETAL DISEASES RESEARCH AND TRAINING PROGRAMS

The Musculoskeletal Diseases Program supports basic and clinical research on the various components of the musculoskeletal system in normal and diseased states. Utilizing funding mechanisms such as regular research grants, career development awards, training grants, and contracts, the budget for these programs was approximately 16.4 million in FY 81, supporting nearly 200 active grants. However, budgetary constraints and inflation, have led to funding of a limited number of the most meritorious projects.

The training and manpower development aspects of the program have increased slightly during the past year. The Clinical Investigator Awards are maintained at a constant level with little encouragement for increased numbers in future years. In general, the training profile continues to be a positive indicator for the development of future researchers.

Workshops and conferences in currently important topics represent a unique opportunity to exchange scientific results and stimulate new research. All conferences have been sponsored in collaboration with other Institutes of NIH or private organizations.

The major meeting of FY 81 was a Workshop on Idiopathic Low Back Pain. From 50 to 80 percent of the population report experiencing some low back pain. The total economic burden of this problem exceeds $10 billion. Scientists from several disciplines presented papers and held discussions on five aspects of low back pain research: epidemiology, anatomy, biomechanics, biochemistry, and neuromechanisms. Five panels were convened to summarize the key points of each section and propose directions for future research. A book and journal article will be published as the result of this workshop.

A conference was held on the organic matrix of mineralized tissues, where the production of and interrelationships between collagen, proteoglycan, and other proteins were discussed. Researchers exchanged views about the role of these organic substances in the mineralization and remodelling processes. As in past years, a Gordon Conference on Bone and Teeth was supported to promote interchange among leading scientists. Osteoporosis was the subject of a large symposium concerning the etiology, epidemiology, diagnosis, and current treatment of this metabolic bone disease.

A new contract was initiated to assist in the management and facilitate supporting services for an Evaluation of the Musculoskeletal Diseases Program. Panels of scientific and management experts will evaluate the past performance of the program and recommend revised goals and objectives to improve future program direction. The evaluation will require about two years to complete.

Basic Bone Research

The bone research component of the Musculoskeletal Diseases Program includes projects on the growth, structure, metabolism, and mineralization in both normal and abnormal bone. Basic studies form a foundation upon which clinical advances will develop in the future.

Many scientists are studying various aspects of the growth and structure of bone. At Pennsylvania State University, research is progressing on the role of carbonic anhydrase which occurs in large quantities in osteoclasts during resorption and calcification. It is possible that carbonic anhydrase inhibition could be used in treating osteoporosis.

Investigators at Montefiore Hospital in New York City are exploring the structure of epiphyseal growth plate cartilage. Using improved methods and sophisticated immunoelectronmicroscopy, they have provided for the first time direct evidence in support of aggregation of cartilage proteoglycans in the extracellular matrix. To unravel the process by which cartilage can be mineralized into bone, this group is also studying link protein from proteoglycan aggregates. These properties of link protein are of interest because proteoglycan aggregates inhibit mineralization and chondrocytes from the aggregates can be modified to abolish the inhibition of mineralization.

A University of Pennsylvania grantee is exploring the optimal electrical parameters for stimulation of growth in the epiphyseal plate. The study has shown that CAMP acts on chondrocytes in the growth plate by stimulating proliferation and depressing differentiation. This study can be useful in treating children with congenital abnormalities or trauma causing unequal leg lengths.

A grantee from Children's Hospital Medical Center in Boston has shown that the earliest solid phase Ca-P deposits into cartilage are in the form of brushite ($CaHPO_4 2H_2O$) and only later when there is increased maturation is there deposition of poorly crystalline hydroxyapatite. Changes in x-ray diffraction patterns which occur as a function of maturation are probably not due to an amorphous phase as previously thought but more likely due to crystallinity of hydroxyapatite and to the presence of brushite.

Several new basic bone studies are being initiated. One investigation will attempt to characterize and isolate a growth factor from rat calvaria which stimulates bone DNA and collagen synthesis. Another grantee will look at the effects of hormones, especially insulin and $1,25(OH)_2D_3$, a metabolite of Vitamin D, on bone collagen metabolism. A third study will synthesize fluorinated diphosphonates and test these compounds as inhibitors of bone resorption for use in disorders such as Paget's Disease.

The Musculoskeletal Diseases Program provides extensive support for basic studies on the relation between bone metabolism and mineralization. A researcher from the University of Arizona is studying the actions of $1,25(OH)_2D_3$ on target cells in kidney, pancreas, pituitary and mammary glands. This work helps characterize the mechanism of action of steriods in controlling gene expression and the active transport of calcium. It can lead to treatment for renal osteodystrophy, hypoparathyroidism, osteoporosis and vitamin D resistant rickets.

A few years ago researchers discovered a protein in bone (osteocalcin) which contains a vitamin K dependent calcium binding amino acid, γ carboxy glutamic acid (Gla). Since then numerous groups have studied its function and metabolism. The group at Children's Hospital Medical Center in Boston has discovered that (1) a precursor of osteocalcin exists, (2) the Gla level in pathological conditions is ten fold that of normal which suggests a fundamental role of Gla protein in pathologic deposition of calcium and (3) the Gla content of rachitic bone was higher than normal in unmineralized osteoid and approached normal in mineralized bone. Using a sensitive method for measuring Gla in urine, this group has shown high levels in children which decrease with age. However, in osteoporosis, the levels are elevated.

At the University of California, San Diego, a scientist has devised a radio-immunoassay for human bone Gla. Studies show the highest levels of this protein in Paget's Disease, hyperparathyroidism, osteodystrophy and osteopenia. It is a useful tool in the biochemical assessment of the skeleton. Also shown was that Gla, which plays a role in fracture repair as well as bone structure, has its synthesis regulated by $1,25(OH_2D_3)$. This is the first bone specific protein whose synthesis is increased by an active metabolite.

The role of the matrix vesicle (MV) in calcification is an important step in mineralization. A group at the University of South Carolina has discovered matrix vesicles are devoid of mitochrondria, lysozomal and endoplasmic reticular enzymes, and possesses characteristics of plasma membranes. Differences in amount of phospholipid of the vesicles suggests modification of membrane components during formation. The mineral content of MV's is labile and vulnerable to release which causes a reduction in calcifiability Alkaline phosphatase acts as a binding agent or transport protein. Gla is not involved in MV mineral loading.

A grantee from Washington University in St. Louis studying circadian variations found deposition of mineral in MV usually occurs 12 hours after production of matrix. Cartilage matrix formation appears during the day; mineralization usually peaks at night. This explains difficulties demonstrating MVs in cartilage undergoing mineralization.

A group from the University of California, San Diego, while studying the effects of gonadal steriods on calcitonin secretion showed that normal females are calcitonin deficient compared to males and both sexes show an age-related decrease in this hormone. These findings could play a role in the etiology and pathogenesis of age and sex-related loss of bone mass in humans.

Acquired and Inherited Bone Diseases

Many investigators are focusing clinical research directly on one or more particular disease. All aspects of a disease process may be considered, such as etiology, diagnosis, prevention, and treatment.

The Musculoskeletal Diseases Program prepared and distributed a Request for Applications on a Clinical Trial of Fluoride in Osteoporosis. Two grantees were awarded for studies of approximately 200 patients each. Based on fracture incidence in populations matched for age and sex, studies should yield statistically valid safety and efficacy data on this fluoride therapy.

Other studies on osteoporosis are also being supported. An investigator at Creighton University is in the fifteenth year of a 20-year longitudinal study of females in the peri-menopausal period. Results will not be available until the completion of the study. However, current correlatory studies indicate calcium absorption is normal in this population. A grantee at Texas Women's University has recently completed a 20-year follow-up survey of individuals by comparing bone density, diet histories and biochemical determinations. Preliminary results show that in the female there is a significant positive correlation between bone mass and original dietary intake of protein, calcium and phosphorous. No similar correlation exists with the present diet.

An epidemiological study is underway at the Mayo Clinic relating bone loss during aging with fracture incidence. Studies show mean bone density is lower in patients with osteoporosis than in age and sex-matched normals. By 65 years, half the normal women have bone mineral values low enough to be classified as osteoporotic.

An investigator at the University of Pittsburgh is assessing bone loss via CT scanning and correlating it with life style. The purpose is to determine if increasing exercise retards onset of osteoporosis. A grantee from Indiana University is looking into skeletal abnormalities in diabetics. Preliminary studies show that diminished bone mass is inversely related to the duration of the disease. These results suggest that impaired endosteal cortical growth may be the cause of the decreased bone mass. At Washington University in St. Louis, a grantee has a new diabetic rat model which relates closely to the pattern of disease growing children who have insulin dependent diabetes mellitus in regard to bone metabolism.

Two other acquired bone diseases are being investigated. A grantee from Duke University is studying x-linked hypophosphatemic rickets and delving into the abnormality of vitamin D metabolism as a pathologic factor related to impaired renal tubular reabsorption of phosphate.

Another investigator from Children's Hospital in Philadelphia is looking into vitamin D therapy in renal osteodystrophy of children. Pilot studies show a beneficial effect of $25(OH)D_3$, a vitamin D analog, upon linear growth, parathyroid hormone and bone histology of children with the disease. Additional studies imply it may take a combination of vitamin D metabolites to heal the complex bone abnormalities.

Several inherited bone diseases are being explored through research funded by NIADDK. They include osteogenesis imperfecta (OI) and Ehlers Danlos as well as genetic bone dysplasias. A Children's Hospital Medical Center (Boston) researcher found that in Ehlers Danlos sub Type II hydroxylysine, an amino acid restricted to collagen, is almost missing in skin collagen and reduced in other tissues. This abnormal linking in collagen is thought responsible for the weak connective tissue. In addition, two defect variants are known in Ehlers Danlos: (1) defective structural gene from proα 2 collagen and (2) defective N-terminal pro collagen peptidase. These defects are important for prenatal diagnosis of the disease and to help unravel the normal biology of collagen. In their studies of OI, the Children's Hospital group discovered that bone from the tibia of an OI patient contained two to three times the hydroxylysine content of controls. This finding indicates a defect in type I collagen. An investigator from Johns Hopkins University is delving into the genetic heterogeneity of osteogenesis imperfecta. These studies support the hypothesis that there are two dominant OI syndromes based on the presence or absence of dental abnormalities. There is no increase in frequency of stillborns or interuterine fractures in dominately inherited OI. Individuals with OI are taller than average. Also, hearing loss, if present, in younger individuals is conductive but becomes sensorineural in later life.

Osteoarthritis and Joint Replacement

Normal and diseased cartilage is being studied to elucidate the osteoarthritic process. Research continues on both biochemical and biomechanical phenomena and the interrelationship between them. The biomechanical investigations measure and model joint forces, geometry, properties, and lubrication.

Two studies headed by investigators of Rensselaer Polytechnic Institute are investigating the correlations between mechanical, electrical, and biochemical effects on cartilage. Cadaver and animal tissue samples, either normal or diseased, are utilized. Stress and stress rate under uniform strain are influenced by ionic concentration in the surrounding medium. Internally generated electromechanical forces shield matrix charge groups and lead to greater energy dissipation. A new technique permits simultaneous measurement of shear modulus, elastic modulus, and permeability from a single experiment. The shear properties of cartilage can now be correctly attributed to the biphasic collagen and proteoglycan interaction.

Other mechanical testing of cartilage is based on force and pressure measurements on intake whole joint. Promising work at the University of Pittsburgh uses flexible wafer-thin pressure transducers inserted into fresh cadaver knee joints. Researchers at Beth Israel Hospital in Boston have developed unique viscoelastic and shear measuring capabilities for cartilage. Large differences exist in shear properties from normal and fibrillated cartilage areas. The decrease in shear modulus occurs before biochemical changes (e.g. uronic acid) can be noted. Investigators from the West Virginia University have proposed a two-phase etiology for osteoarthritis. First, fribillation is initiated over areas where there are gradients of high stiffness in underlying bone; then the disease progresses primarily in areas whth high stiffness subchondral bone. This theory was demonstrated in sheep which developed osteoarthritis subsequent to walking on concrete runways.

Biochemical studies deal with the production and degradation of cartilage tissue. Many of the degradive enzymes and prostaglandins assumed to be contributory to osteoarthritis are endogenous to normal cartilage cells. At Rush-Presbyterian-St. Lukes Hospital in Chicago, an in-vitro chondrocyte culture was established that did not permit phenotypic alteration. Lysozyme and collagenase were produced in this isolated environment. Investigators have proposed a positive feedback cycle of prostaglandin release inducing further release of prostaglandins as a mechanism to explain the chronicity of arthritis episodes.

Joint replacement research is often directly related to studies on locomotion and osteoarthritis. Many of the mathematical analyses and experimental models of joints are scientifically relevant in normal, diseased, and replacement status.

Diagnosing the condition of a joint before and after replacement surgery is important to proper clinical management of patients. At the Mayo Clinic a mechanical knee testing apparatus has been utilized on 200 artificial knee patients. Replacements with patello-femoral components were found to perform better than those without them. These results were substantiated by gross clinical changes occurring later. In general, the automated measurement and analysis system was judged more accurate than routine clinical examination. Other investigators have demonstrated that as compared to normals, knee prosthesis patients have shorter stride length, less knee flexion and abnormal torque about the knee and ankle. Recent experimental procedures developed at the Hospital for Special Surgery in New York may allow acoustic emission tests to identify early loosening of implant devices. Preliminary tests of this device have identified large differences between well fixed and loose hip replacement implants.

Joint loosening remains the leading cause of implant failure. Investigators at UCLA always observe a cellular response at the bone-cement interface. With good penetration, a fine membrane with macrophages appears, but with poor penetration, the typical result is a thick fibrous membrane with eventual loosening. Bone ingrowth without cement was successful with sintered cobalt beads but not with other porous materials tested. A new study at the University of South Carolina has demonstrated good shear strength and early bone in-growth with porous plastic coated prostheses. Another researcher has shown that pre-coating the prosthetic stem with cement yields a simplified surgical procedure and improved strength.

Infection and toxic reaction to implants and wear products are potential causes of long or short term failure. At the University of Florida, scientists have determined the minimum quantities of bacterial infiltration that will lead to infection and eventual loosening. Other investigators are tracing the path of intermediate organo-metallic compounds as they proceed from the implant site to other body organs. A study at Rush-Presbyterian-St. Lukes Hospital in Chicago has shown a high rate of tumorgenesis with one metallic alloy, MP 35N. Additionally, they developed new methods for trace metal identification. The improved technique indicates less concentration of trace metals than previously reported.

At the University of California, Davis, a relatively simple test for metal allergy has been developed. The leukocyte migration test shows about 24% of patients are sensitive although not all are highly reactive. Upon implant removal, many reactions were shown to be reversed. Thrombus formation is a frequent complication of surgery for hip replacement. A recently initiated study at Temple University in Philadelphia will measure the subtle and gross vascular changes such as increased permeability and excessive vasodilitation.

Fracture Healing

Research on bone healing encompasses both basic and applied science. Grantees from the Hospital for Special Surgery in New York are looking into biochemistry of the healing process. Their research of healing callus shows: (1) an early hexosamine rise represents proteoglycan formation; (2) high Ca/P ratio followed by apatite formation indicates binding of calcium to proteoglycan; and (3) changes in phospholipid content are most abundant during mineral accumulating activity.

A group at the Mayo Clinic is conducting several projects on the transport of substances into bone. Studies in canines show that insertion of methyl methacrylate inhibits fracture healing, decreases blood flow and increases the incidence of infections. Fractures fixed with plates vs. medullary rods indicate slower healing occurred on the rod side. Indicator dilution techniques revealed the mechanism of increased blood flow to fracture site is due to recruitment of capillaries and cells of bone.

Another study at Mayo shows that bactericidal concentrations of antimicrobial agents must reach intersitial fluid space, which is the site of drug-organisms interaction. Interstitial fluid concentration of antibiotic in both normal and osteomyeletic bone may be measured via serum assays. Lastly, a blood-bone barrier does not exist. B-lactam and amino-glycoside antibiotics can cross the capillary membrane in normal and osteomyeletic osseous tissue.

Bone mechanical loading and biological remodeling are under study by a Massachusetts General Hospital group. Several characteristics of bone discovered by this group are: (1) human cortical bone has poor fatigue resistance; (2) monotomic fracture behavior and fatigue fracture are controlled by strain rather than stress; (3) bone fatigue damage accumulation is physiologic rather than pathologic; and (4) bone loss is sensitive to small changes in bone stress.

Electrical stimulation devices for clinical use in fracture management for nonunions, failed unions, and psuedo-arthrosis were approved by the FDA last year. A group at Columbia University, utilizing external electromagnetic stimulation coils, has shown that immobilization and non-weight bearing increases the likelihood of successful healing on adults. In studying radiographically this repair process, it appears that the early "clouding" of the gap region is probably related to mineralization of bone flanking the gap and to active bone resorption associated with vascular invasion of the gap.

Studies at the University of Pennsylvania, exploring the basic biology of electromagnetically induced current, found that the levels previously effective in inducing osteogenesis do not produce significant increases in stiffness in stimulated callus. Their device consists of a trancutaneous cathode at the fracture site and a skin anode several inches away. They studied the changes in the medullary canal cells in the vicinity of the cathode. There was initial calcification along the collagen fibrils and in lipid bodies in the matrix. In addition, they found stress generated potentials in callus as early as four days following fracutre.

Investigators at the University of California, Riverside, studying electrical stimulation at the cellular level discovered osteoblasts grown in the presence of pulsed electro magnetic fields noticeably increased collagen synthesis and cell proliferation. The effects of parathyroid hormone (PTH) and osteoclast activating factor (OAF) were abolished by the electrical stimuation but effects of $1,25(OH)_2D_3$ were not changed. These data suggest a membrane localized mechanism for electrical fields.

MULTIPURPOSE ARTHRITIS CENTERS PROGRAM

Overview

The Multipurpose Arthritis Centers Program is currently at a level of twenty-three Centers located in eighteen states. The NIADDK initiated the Arthritis Centers Programs in Fiscal Year 1977 with awards totaling $2.5 million to 15 institutions. Awards were made to nine additional Centers in 1978 and 1979 and one new Center in 1980. In Fiscal Year 1981, the Institute directed approximately $7.6 million toward the Centers active during the year. The average size of an Arthritis Center during Fiscal Year 1981 was approximately $363,000 total costs.

Activities in the Centers

The Arthritis Centers conduct activities in three areas: basic and/or clinical research, educational activities and community demonstration projects. Most of the projects underway could not have been started without the interaction of individuals brought together under the aegis and support of the Arthritis Center grants. Examples of major efforts of the centers include research into the genetic basis of arthritis; new advances in health services research, such as cost-effectiveness studies and analysis of disability; comprehensive education of allied health professionals; and research into the best methods of delivering arthritis health care to those least able to contain it, i.e., the home-bound individual and the resident of the inner city. In addition, many of the Centers have programs dealing with problems of arthritis in children, a particularly tragic form of the disease.

Research Component Activities

It is expected that a Multipurpose Arthritis Center will have a strong component of biomedical research. Center grant support is intended to complement traditional research grant support in a given institution in order to establish related special projects and stimulate the development of new research projects. Consequently, each potential Multipurpose Arthritis Center is expected to be receiving research grant support for basic and/or clinical biomedical research related to rheumatic diseases as a prerequisite for center grant award.

Research projects supported by center grant funds are almost exclusively developmental and feasibility studies. These studies are designed to encourage investigators to explore interdisciplinary and highly innovative scientific approaches which may later form the basis of applications for traditional research grant awards from the National Institutes of Health or other agencies.

As part of a comprehensive study of the genetics of the rheumatic diseases, the center located at the Johns Hopkins University in Baltimore is attempting to determine predisposition to various rheumatic diseases in two highly inbred populations: the Old Order Amish and the Mille Lacs Band of Chippawas. The Amish are an especially appropriate group for studies of this nature since they are a genetically closed population (anyone marrying outside the Amish faith is excommunicated). Their gene pool is also relatively small since the entire population originates from only about 200 individuals who came to the United States in the eighteenth century. Extensive genealogical histories are also available. The diseases being studied include rheumatoid arthritis, the spondylitic disorders and psoriatic arthritis. The aims of the project are: (1) to elucidate the genetic mechanisms responsible for the susceptibility of given populations to various rheumatic disorders, and (2) to investigate any environmental factors that may explain the increased frequency of the diseases in these populations. The researchers are particularly interested in studying antigens -- substances that stimulate the production of antibodies by an organism's immune system. Initial immunologic studies have shown that a specific, genetically determined antigen (HLA-B27) occurs at a frequency of about 22 percent in the Chippawas but only 2.2 percent in the Amish. Significantly, the distribution of rheumatic diseases is different in both these populations. These genetic studies represent a unique resource, since inbreeding in human populations is uncommon and observations over many generations is extraordinarily difficult, if not impossible. Thus, populations with a small gene pool and limited environmental variability could yield important information about the association between antigens and rheumatic diseases.

Other research on the association of genetics with susceptibility to rheumatic diseases is underway at the University of Alabama Multipurpose Arthritis Center in Birmingham. Studies center on antigens, particularly "DR antigens," which are found on the surface of certain types of immune cells. Individuals possessing these antigens appear to have an increased risk of developing some rheumatic diseases. As a first step in proceeding with these investigations, the center is attempting to purify these antigens and produce antibodies against them. Once this is completed, it may be possible to classify these antigens with greater specificity and find some that are directly associated with specific rheumatic disorders. In addition, the purified antigens will be used to study the interactions of cells in the immune system and the role they play in disease susceptibility.

The center at the Indiana University School of Medicine conducts basic investigations into many aspects of degenerative joint disease (osteoarthritis), with special emphasis on the mechanisms of articular cartilage destruction and repair. Researchers hypothesize that dysfunction of the sensory articular nerves may lead to joint damage as a result of the loss of protective muscular reflexes. To test this hypothesis, they surgically cut the major afferent nerves to the knee of dogs and subsequently observed biochemical changes typical of early osteoarthritis in the animals. More long-term studies are now underway to determine if nerve transection also leads to morphological changes associated with the disease, or if it is merely limited to transient biochemical alterations.

Clinical studies are also being conducted at several centers. The center at the University of Michigan in Ann Arbor is involved in measuring the binding of the steroid prednisolone to plasma in the serum of patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), as well as in normal volunteers already being treated with the same drug. Prednisolone and related drugs are commonly used in the treatment of these diseases. Taking blood samples timed from the administration of the drug to RA patients, researchers found a large interpatient variation in the plasma protein binding of prednisolone, ranging from 66 to 93 percent bound. Variable binding was also observed in SLE patients taking prednisolone. Analysis of data regarding the relationship of this variability to the efficacy and toxicity of chronic prednisone therapy in these patients is currently underway. Initial results suggest that individualization of drug regiments will be important in planning therapy of patients with rheumatic disease.

The center located at the Robert Breck Brigham Hospital in Boston is trying a new experimental approach in the treatment of especially severe rheumatoid arthritis. Although rheumatoid arthritis can usually be treated by a number of drugs, such as aspirin and other anti-inflammatory drugs, some cases are totally resistant to these therapies and thus potentially life threatening. The stronger drugs, e.g. penicillamine and cytotoxic drugs, used to treat these resistant cases of the disease have undesirable side effects, such as anemia and skin eruptions. A new approach, used in a small-scale clinical study, is total irradiation of the lymph nodes of patients who have been unresponsive to other forms of therapy. The rationale for this therapeutic method is based on the immunosuppressive effects of radiation. Nodal irradiation may produce a regression of the arthritis by eliminating immunocompetent lymphocytes. Patients will be carefully selected to assure that all conventional means of therapy have been exhausted. In addition to the potential for treating previously intractable cases of rheumatoid arthritis, this study might yield information on the role of the lymphocyte in the pathogenesis of rheumatoid inflammation.

A major new thrust of the Multipurpose Arthritis Centers is health services research. An example of this activity is a project soon to be initiated at the center located at the Robert Breck Brigham Hospital in Boston. The aims of this project are: (1) to determine the cost effectiveness of joint replacement surgery in individuals with arthritis impairment; (2) to document changes in function and health status in patients who have had joint replacement surgery; and (3) to attempt to identify prognostic variables for successful joint replacement surgery. Over 120,000 joint replacement operations are performed annually in this country at an estimated cost to society of approximately $840 million. Although reduction in pain and improvement in joint function as a result of this surgery is well documented, what is not clear is the extent to which surgery corrects the person's handicap. Psychological factors and debilitating changes in areas of the body not affected by the surgery may complicate post-surgical rehabilitation and result in only partial relief of symptoms. The results of this study may help to provide knowledge about the long-term benefits of joint replacement and the variables that predict successful surgery.

The center at Boston University decided to devote a major effort to developing a more useful method to evaluate the impact of arthritis programs on health status. The result of this effort was the development of a self-administered instrument called the Arthritis Impact Measurement Scales (AIMS), which includes cales for mobility, physical activity, social activity, social role, dexterity, pain, activities of daily living, depression, and anxiety. The instrument has already been presented at national meetings of the American Rheumatism Association and the American Federation for Clinical Research and has been published in the journal, Arthritis and Rheumatism. The AIMS instrument will be useful in assessing and comparing health status in individuals and populations with connective tissue disease and should be an advance in the ability to measure the impact of new therapies and programs.

Education Component Activities

Consistent with the National Arthritis Act and the Arthritis Plan, a major feature of the Multipurpose Arthritis Centers Program continues to be educational activities. Many ongoing projects are intended to improve rheumatic disease care at the level of the primary care physician. An example is the Dial-Access System (DAS), a program that has been under way for the last three years at the Alabama Multipurpose Arthritis Center. The DAS is a method of medical consultation by means of tape-recorded messages that are accessible on request through long-distance toll-free telephone lines. The subject matter concerns the most recent diagnostic and therapeutic information on specific arthritis and rheumatology problems. Over 100 different tapes have been produced so far, ranging from descriptions of the more common rheumatic disorders (such as rheumatoid arthritis and systemic lupus erythematosus) to the interpretation of laboratory tests and recommendations for drug therapy. During the first two years of the grant to this center, over 24,000 calls were made to the DAS. In a typical month, over 1,200 calls were made to the DAS from 41 states. Survey questionnaires have shown that most of the calls came from physicians in family practice, general surgery, internal medicine and orthopedic surgery, more than half of whom practiced in communities with populations less than 100,000. These survey results are indicative of the usefulness of this program in areas that may be located far from major medical centers, where rheumatological consultatation is easily obtained. There are plans to add another one hundred tapes to this system in the next several years.

Other areas of educational emphasis in the centers relate to allied health professionals (AHP). At the center at the University of California, San Francisco, for example, staff members have been able to provide inservice education for numerous health care agencies in the center's service area. Most presentations are given by both a nurse and a physical therapist with special emphasis on providing educational activities for those working in the area of home care. In one home care agency, four groups of 15 to 20 home care aides each received three hours of teaching. In another similar agency, 120 aides received two hours of instruction each. The presentation by the nurse dealt with the disease processes of rheumatoid arthritis and osteoarthritis, with a summary of available treatments. The physical therapist described the use of heat, cold, and exercise, and gave an introduction to the subject of joint protection.

The center at Indiana University School of Medicine provides a prototypic approach to educating physicians. Center staff provide second-year medical students with a series of lectures correlating basic science knowledge with clinical information. These lectures include videotapes, radiographs, and demonstrations of history-taking and physical examination. Small groups of students under the supervision of a senior rheumatologist or a rheumatology fellow examine patients with rheumatic disease. For junior medical students, there are lectures on specific disease entities, and opportunities to be ward clerks on the rheumatology service at the university hospital. A one-month rheumatology elective is available for senior students. This includes participation in the care of both in- and out-patients, exposure to the team concept of care (including the nurse, occupational therapist, physical therapist, and social worker), and formal lectures. Students taking this elective are expected to become proficient in common, practical aspects of treating chronic rheumatic disease. This includes history taking, joint examination, the proper use of drugs, diagnosis of commonly encountered diseases, and follow-up of the patient with rheumatic disease. In addition to the attention given to medical students, residents and interns of medicine, surgery, and orthopedics may take rheumatology rotation at the hospital. There are also less formal conferences and teaching sessions held on a regular basis for all medical personnel including the house staff and attending physician.

Community Component Activities

Community projects during the past year have included studies of model health care delivery for the home-bound arthritis patient and the resident of the inner city; the practical utility of upgrading the care provided by the community physician; and programs to facilitate the use of the local Multipurpose Arthritis Center as a community resource.

As part of its community commitment, the center located at the Robert Breck Brigham Hospital in Boston has carried out the first phase of a model health care system for arthritis disability. In the initial effort, the center screened the entire population of a Boston neighborhood to establish a registry of persons with treatable functional disability related to arthritis and other musculoskeletal problems, and to determine their health care and social needs. Using multi-lingual surveyors, the center made telephone contact with 3,800 of the approximately 8,500 adults living in three census tracts of the Jamaica Plains area. Questionnaires were mailed to 600 of those respondents who indicated some form of joint-disease related handicap in order to document their handicap and need for specialized health care. A rehabilitation team subsequently examined individuals who met the criteria of the American Rheumatism Association for disability (that is, "handicapped by discomfort or limited mobility in one or more joints"). This assessment included any necessary occupational or physical therapy and referrals to specialists. An "arthritis advocate" provided information about related health services and will also monitor the progress of this study. The survey revealed a number of previously unidentified arthritic conditions such as psoriatic arthritis and gout, which are easily treatable. The survey also disclosed that patients had little knowledge about rehabilitation, joint replacement surgery, or available resources. In the coming year, with the completion of the survey, the effectiveness of the advocacy services will be tested. Some individuals will receive "intensive advocacy," while others will be in an "information only" group. For the former group, the advocate will do everything possible to be of assistance, from providing education about the rheumatic diseases to giving assistance with transportation. The other group will receive printed information about their diseases, although any request for further assistance will be honored. Evaluation of the program will occur through means of an assessment of any increase in functional capacity of the patient, health services utilization, and patient satisfaction. A study of this nature is significant because community programs in other categorical diseases (such as hypertension, diabetes, and cardiovascular disease) have successfully reduced hospitalizations and costs. If the same sort of improvement is noted with respect to the rheumatic diseases, this may prove to be a useful adjunct to the management of arthritic disorders in the community setting.

In a study begun several years ago, the center at the University of Michigan began to identify "educational influentials" in several communities. Such individuals are physicians who command the respect of their colleagues and have a position of influence with respect to the medical care delivered in their communities. The goal of the center was to provide increased training in the rheumatic diseases for educational influentials, in order to upgrade the care of arthritis patients in the community. Initial work on this project documented the level of care provided to patients with rheumatoid arthritis in small communities. Preliminary measures of activity already indicate that there is a favorable change in treatment patterns taking place as a result of the existence of educational influentials trained in rheumatology by the Michigan center.

The center at the University of Tucson is examining the adequacy of building codes with respect to the physical disability of patients with arthritis. In a comprehensive effort utilizing not only biomedical engineers, but also physical therapists and occupational therapists, permanent equipment has been designed and fabricated to determine the optimum position and design of ramps, doorknobs, handrails, light switches, and floor surfaces. Over 250 individuals have been studied so far, with several hundred separate observations being made on each. The center is collaborating with the National Bureau of Standards to further this study on a nation-wide level.

Arthritis in Children

Many of the centers are active in studies and programs dealing with arthritis in children -- a form of the disease estimated to affect over 250,000 in this country alone.

In a project started over two years agao, the center at Stanford University began a retrospective study designed to identify medical and social factors that may have influenced the outcome of juvenile rheumatoid arthritis. At the present time, 267 patients have been recruited for the study and have filled out questionnaires relating to the course of the disease and the medical and surgical interventions they received. This study is especially significant since no data are currently available concerning how juvenile rheumatoid arthritis affects the lives of patients. Thus, it is not now possible to provide appropriate education and social care for the child with arthritis and his or her family. Once the factors affecting the subsequent development of the patient are identified, it is conceivable that those interferring with achievement of adult independence can be ameliorated. Another goal will be to correlate the school experience of the child having arthritis with the adult outcome of the disease. Factors that seem to affect the adult outcome adversely will then be identified and can be corrected in the future.

Computers in the Rheumatic Diseases

A noteworthy development during the past several years has been the utilization of computers in all phases of center activities, ranging from instruction to clinical research, to the use of the computer as an automated clinical consultant.

Computer-based technology has the ability to do more than serve simply as an efficient data storage system. The effective use of this valuable system is amply demonstrated by the Multipurpose Arthritis Center at the University of Missouri in Columbia. Investigators there are applying the techniques of artificial intelligence to construct a computer-based system, which can act as a clinical consultant to the non-rheumatologist physician. During the past two years, clinical criteria have been developed for seven rheumatic diseases: mixed connective tissue disease, systemic lupus erythematosus, progressive systemic sclerosis (scleroderma), polymyositis, rheumatoid arthritis, Raynaud's and Sjogren's Syndrome. The center has also prepared a patient data entry form, which permits the entry of raw numerical data, as well as routine clinical indications and symptoms. So far, the computer system has been able to consistently determine the correct diagnosis (as compared to a consensus of rheumatologists) in over 93 percent of the cases examined. The center is now in the process of adding about 40 more diseases to those already included in the computerized system. Once these disorders have been added, the system will include about 85 percent of all major rheumatological diseases encountered. This effort is quite important: It has been estimated that only about three percent of arthritis patients are ever seen by a rheumatologist; an "automated rheumatology consultant" would be able to benefit those patients of the general practitioner by providing more expert diagnosis and treatment than they would ordinarily expect to receive. Even for the rheumatologist, the automated system could have an educational benefit. The protocols developed here may also privide a model system for other fields of medicine which may wish to produce their own automated systems. In addition to integrating new diseases into the system, the center plans to expand the capabilities of the system to consider patient prognosis, management, and eventual treatment, as well as diagnosis, during the coming year.

SPECIAL PROGRAMS IN ARTHRITIS AND RELATED DISEASES

The following important activities are being developed to address certain specific features of the National Arthritis Acts of 1974 and 1976, and several recommendations of the Arthritis Plan submitted to the Congress in April 1976 by the National Commission on Arthritis and Related Musculoskeletal Diseases.

EDUCATION PROGRAMS

A major goal of the Arthritis Education Programs Office is the maximum use of the tools of communication to inform and educate allied health professionals about arthritis. Significant progress has been accomplished towards this goal, and stimulating and assisting the development of public, patient and professional education as essential elements in achieving optimal treatment and management of arthritis.

Arthritis Information Clearinghouse Office

A major component of the Education Programs Office is the Arthritis Information Clearinghouse. Established by the NIADDK following authorization by the National Arthritis Act and recommendation by the National Commission on Arthritis and Related Musculoskeletal Diseases, the Clearinghouse is completing its third year of operation aimed at improving the lines of communication among the health professionals serving the arthritis patient. The major function of the Clearinghouse is to collect, screen, store, and disseminate information about educational materials and programs on the rheumatic diseases.

In serving as a broker to foster the flow of arthritis information, thus helping users to locate and select educational materials, the Clearinghouse refers clients to the appropriate developer or source rather than acting as a distributor of the materials itself.

The Clearinghouse data base is now fully operational, consisting of 1800 records, retrievable by keyword, author, source, publication year, and text words in titles and abstracts. Included in the data base are print and audiovisual materials, journal articles, reports and textbooks, among other items. Inasmuch as the data base is in place and the mechanism for acquiring and processing materials are well established, no further development in this area is required at this time. A cumulative catalogue of 1980 accessions has been produced to supplement the 1979 catalogue. Printed and audiovisual materials are being added to the data base as they appear; since retrospective searching has been completed, the rate of acquisitions in these areas should level off or decline. An increased effort to identify and describe educational programs and incorporate them into the data base is one major priority for the coming fiscal year.

Products and Services

Clearinghouse products thus far include more than 30 bibliographies and catalogues, a Thesaurus, and a cumulative catalogue of accessions, while its services, approximately 400 per month, include requests for publications, mail list additions, data base sources and referrals.

Due to a heavy demand of requests for literature searches, the data base has grown rapidly. In order to streamline requests, packaged bibliographies on frequently requested topics have been prepared. Several directories have been assembled to speed answers to information and referral questions. Approximately 16,000 Clearinghouse products have been distributed during the past fiscal year.

Outreach

The Clearinghouse has an active outreach program, contacting conferences and meetings and providing exhibit materials and package enclosures. A short slide talk explaining Clearinghouse activities has been presented at some meetings.

As part of its outreach program, two Arthritis Information Clearinghouse Bulletins have been published this year to alert users and health professional constituents as to new developments. However, due to Federal budget constraints, plans for further Bulletins have been set aside for the time being. Instead, a less expensive but equally effective memorandum will be used to maintain communication with Clearinghouse users.

In addition, two of the Clearinghouse exhibits have been sent to meetings and conferences, including the annual meetings of the American Rheumatism Association and the Arthritis Research and Education in Nursing and Allied Health, as well as the Medical Library Association Meeting, and a number of regional arthritis-related meetings.

Cooperation with Multipurpose Arthritis Centers

The Clearinghouse is continuing its efforts to collect information on community and institutional patient education and in-service training programs relating to arthritis. Since many of the Multipurpose Arthritis Centers are emphasizing team care and developing programs and resources to implement education programs, there was a need to develop a method for the Clearinghouse to identify MAC efforts in this area and incorporate knowledge into the Clearinghouse data base.

A number of possible alternatives have been explored to strengthen communication between the Clearinghouse and the MACs. For example, the Clearinghouse will be a depository for MAC reports, reprints, and studies. Curricula and program descriptions of patient education or in-service training classes are being collected, and materials developed such as syllabi, resource lists and supplementary materials are also being sought.

In the next fiscal year, the Clearinghouse will continue to provide direction and assistance to education activities within the centers program, and will continue to conduct needs assessments and to identify gaps in available materials. Since both the Clearinghouse and the MACs exist through NIADDK funding, Clearinghouse users assume a close communication link between the two programs.

Advisory Group

An advisory group comprised of eleven rheumatologists, educators, library scientists and other health professionals has continued to help the Clearinghouse in the effective accomplishment of goals. The group, meeting twice yearly, acts as a sounding board and provides recommendations to the Clearinghouse project officer and staff. During the past year, in normal rotation of members, two new advisors have been added, the Head of the Reference Section of the National Library of Medicine, and the NIADDK Information Officer.

Appraisal and User Satisfaction

Inasmuch as the bibliographic information services have matured and become operational, and a host of products and services have been produced after two years of operation, it had been evident that an evaluation or appraisal was needed to determine the effectiveness of the program. To help evaluate services, users have been asked to return a User Satisfaction Form enclosed with mailed materials. Most users have found services very helpful and are satisfied with the response times.

It is interesting to note that consistently almost a third of the forms are returned, a very high response inasmuch as it is difficult to motivate users to return such forms; they take time and the users' own postage.

Unsolicited letters have also been received which relate that the Clearinghouse products, services, and activities have saved physicians, health educators and other professionals considerable time and effort in educating patients and other health professionals.

ARTHRITIS DATA SYSTEMS PROGRAM

The Data Systems Program of NIADDK fosters systematic acquisition, storage, retrieval, and analysis of information about the rheumatic diseases. Major program effort is concentrated upon assuring validity, confidentiality and comparability of data collected in separate institutions. Identifying and integrating data resources with data needs is a continuing component of program activities.

Two data resources, American Rheumatism Medical Information System (ARAMIS) and the Health and Nutrition Examination Survey (HANES I) follow-up are currently of particular interest to the Data Systems Program Office.

A National Arthritis Data Source

The American Rheumatism Medical Information System (ARAMIS) has continued to enlarge its data bank on patients with chronic rheumatic diseases registered in a longitudinal format. It has expanded to include 14,000 patient entries representing approximately 13,000 patient years of experience. The number of participating investigators and centers has also grown. Among the newest satellites are Miami, with Dr. Ray Altman, Principal Investigator and the Multipurpose Arthritis Center at Birmingham, with Dr. Randy Wilson, Principal Investigator.

While it is recognized that ARAMIS is not a substitute for careful research design and execution, it has received increasing recognition as a powerful research auxilliary tool. Its long-range goals continue to be 1) improved diagnostic classification of rheumatic diseases, including establishment of acceptable criteria; 2) identification of "risk" factors associated with good or poor outcomes based on prognostic studies among defined patient subsets; 3) improvement of evaluation instruments for these purposes; and 4) enhancement of the data system to help achieve these purposes.

ARAMIS contributions have been recognized in a number of areas. These include classification studies such as evaluation of JRA and RA criteria, continued work on the scleroderma criteria and development of subset concepts. ARAMIS has supported the examination of prognosis in RA attempting to determine factors influencing outcome. Additional progress has been made in studies of development of patient questionnaires and cost monitoring. Finally, ARAMIS investigators were major authors in the preparation of the ARA Glossary under the chairmanship of Dr. John L. Decker.

Among the studies currently planned at the ARAMIS Core facility are: 1) development of a self-administered computer-compatible questionnaire for arthritis; 2) implementation of a rare disease data bank covering pregnancy in SLE and eosinophilic fasciitis; 3) creation of a registry of patients with rheumatic diseases on immunosuppressive therapy to prospectively monitor the rate of occurrence of malignancies in these patients.

Among the numerous studies planned for ARAMIS satellites over the next three years are:

1) functional outcome and survival in polymyositis/dermatomyositis
2) evaluation to establish the diagnosis and prognosis of scleroderma
3) factors influencing mortality in R.A.

The Health and Nutrition Examination Survey (HANES I) Epidemiologic Follow-up Survey

In cooperation with the National Institute on Aging (NIA), the National Center for Health Statistics (NCHS) and several other Institutes, the Arthritis Epidemiology and Data Systems Program has become active in the design of the instrument for the collection of data in this resurvey of 14,407 individuals who were originally sampled between 1971 and 1975.

Careful preparation of the arthritis section of the follow-up instrument will serve the four principal objectives of the project which are: 1) retrospective morbidity and mortality associated with suspected risk factors; 2) prospective mortality associated with suspected risk factors; 3) changes in patient characteristics between HANES I and current status; and 4) natural life history of chronic disease and functional impairments.

ARTHRITIS EPIDEMIOLOGY PROGRAM

The Epidemiology Program Office is the administrative focus of efforts to encourage epidemiologic research in the field of arthritis and related musculoskeletal diseases. In addition, the office is responsible for facilitating cooperation and exchange of statistical information among independent investigators.

Epidemiologic research activity has increased during the past year in three principal sectors. First, there has been an increment in the number of grant applications with primary emphasis on arthritis epidemiology in the Fellowship and Research Career Programs. Second, there has been growth in the number and sophistication of epidemiologic studies in progress and planned within the Multipurpose Arthritis Centers. Finally, there has been an increase in epidemiology projects planning and funding within the ARAMIS consortium. Two ARAMIS backed population based studies will be included in the 1981-83 program.

Arthritis Epidemiology Conference

The Program continues to convey the information gathered and priorities generated at the Arthritis Epidemiology Conference in September, 1980. A number of publications are in preparation, both in-house and externally, to disseminate conference contents to both the rheumatology and epidemiology communities. These publications include the proceedings with supplemental papers on arthritis data resources and an updated version of the annotated bibliography, Epidemiologic Studies of the Rheumatic Diseases.

In reviewing the Conference Proceedings for further program planning, it is apparent that conferees were in nearly unanimous agreement on six points related to further research:

- A call to avoid prevalence incidence studies just for their own sake, particularly when the expected incidence is low;
- Consideration of very well designed case-control studies;
- Development of longitudinal data sets which follow chronic diseases over a long period of time whenever possible;
- Identification of subgroups and concentration on classification and nosology to a greater degree than before;
- Making maximum use of existing data sets;
- Using disability as one major outcome goal with which to judge our knowledge of these illnesses.

Following the Conference, a number of participants suggested an ongoing workgroup to provide a forum to formulate specific approaches to epidemiologic studies. Plans are in progress to implement this recommendation.

Lyme Arthritis

Studies are continuing on the nature, pathogenesis and etiology of the tickborn disease, Lyme Arthritis. This illness consists of an acute and sometimes chronic arthritis; a characteristic skin lesion; and neurologic and cardiac abnormalities. Currently, prospective studies are focused on patients in order to identify prognostic factors, treatment regimes with antibiotics and long-term sequela. The effort to identify the etiologic agent uses a variety of procedures such as electronmicroscopy of skin biopsy samples, blood cultures, and studies for rickettsia mole.

Ankylosing Spondylitis

Recent studies of Amish famililities noted a high frequency of sacroiliitis and/ or spondylitis. The association with B-27 was quite weak in contrast to findings in a number of other population based studies. Further investigation is planned to attempt to determine what other markers or environmental factors may be contributing to spondylitis susceptibility. Anticipated comparative studies among the three populations of the Amish, the Chippewa Indians and non-inbred patients may stimulate insights into the genetics of this disease.

THE ANNUAL REPORT OF THE DIGESTIVE DISEASES AND NUTRITION CLUSTER

Introduction - Associate Director - Dr. H. P. Roth

The Digestive Diseases Program Area is responsible for the extramural support of grant awards and contracts pertaining to diseases and disorders of the gastrointestinal tract and associated organs. Thus digestive diseases include disorders of the esophagus, stomach, small and large intestines, liver, biliary tract and pancreas. Major digestive diseases include esophagitis, peptic ulcer, diverticulitis, infectious diarrhea, ileitis, ulcerative colitis, and malabsorption, gallstones, cirrhosis, hepatitis, and pancreatitis. The known causes include infections, metabolic disorders, genetic defects, neuromuscular disorders, toxins, and reactions to life stresses.

Digestive diseases constitute a health problem of great magnitude. Of all the causes of disability due to illness in our country, digestive diseases rank second. Over two million Americans are impaired in some degree by these diseases; 1.2 million persons are limited in the work that they can perform and 400,000 are totally disabled. Among the employed, digestive diseases each day account for 200,000 absences from work due to illness, making digestive diseases the leading cause of loss of time from work among male employees. Almost 140,000 veterans receive payments for service connected disability due to gastrointestinal conditions, at a cost to the nation of approximately 100 million dollars per year.

The Digestive Diseases Program continues to respond to the recommendations of the National Commission on Digestive Diseases and is responding to two recent congressional mandates that arose in response to the Commissions recommendations: The Health Program Extension Act of 1980 of the 96th Congress (2nd session) established the "Information and Education Center for Digestive Diseases" and provided for the establishment of the "National Digestive Diseases Advisory Board." A Digestive Diseases Information and Education Center has been established. It is identifying the teaching material already available and preparing additional material as needed. The Digestive Diseases Coordinating Committee is actively working to establish a data base and information retrieval system based upon a standardized definition for digestive diseases as a whole and agreed upon definition for those research areas in digestive diseases which are of special interest.

The NIH Consensus Development Conference "Endoscopy - What is its Role in Upper Gastrointestinal Bleeding?" was held in August 1980. A consensus statement was developed which recognized the value of endoscopy in identifying the cause of upper GI hemorrhage but considered the benefit to outcome of management to be equivocal. The full text of the presentations from the conference are to be published as a supplement to Digestive Diseases and Science.

Representatives of the Intersociety Committee on Human Experimentation in Digestive Diseases met with representatives of other interested groups from within and outside of government at a two day meeting on "Risks of Procedures Used in Human Experimentation in Digestive Diseases". The meeting summarized available information, identified types of information needed and ways of obtaining such information, it particularly focused on the accuracy of data collection. Workshops, conferences and planning sessions, involving the Intersociety Committee and other groups were discussed.

The National Cooperative Gallstone Study group reported on the clinical trial of chenodeoxycholic acid in the medical treatment of gallstones. The drug in the higher dose level was associated with complete dissolution of stones in 14% of cases treated for 2 years.

During this year, the digestive diseases program was divided into 3 units which will provide individual reports: 1. Esophogeal, Gastric and Colonic Diseases, 2. Liver and Biliary Tract Diseases, 3. Intestinal and Pancreatic Diseases.

The nutrition program supports extramural research and training in basic and clinical nutrition. A major initiative in nutrition has been the development of clinical nutrition research units. The directors of these units meet to discuss common problems, to review their experiences and achievements. These units have developed an identity and a focus for nutritional research, bringing together nutrition investigators who might not have otherwise cooperated.

During the FY 1981 there was productive research activity concerned with the basic aspects of the structure and function of the digestive system. However most of the research, was disease oriented, with a focus on topics that were relevant to the elucidation and understanding of diseases process. In nutrition, there was considerable basic research, but a major fraction of the research activity was concerned with obesity. Research on minerals, vitamins and protein and amino acids accounted for the majority of the remaining grants. Of the considerable amount of data and information reported by the grantees in digestive diseases and nutrition during the past year, only a few examples will be cited as representative of the progress that has been made.

Esophageal, Gastric and Colonic Diseases Program Area

(1980 - 1981)

The Esophageal, Gastric and Colonic Diseases Program area is responsible for the extramural support of grant awards and contracts pertaining to the listed organs. The following examples are representative of the progress in this area.

1. Advances in Esophageal Research

The investigations which have been supported by NIADDK in the area of esophageal structure and function have focused on the general problems of esophagitis and the inability of the lower esophageal sphincter (LES) to maintain a tonus adequate to prevent the reflux of gastric contents into the esophagus (a common cause of esophagitis).

A common theory for the production of esophagitis by Gastro Esophageal Reflux (GER) has been that the pressure exerted by the LES, the LESP, was lower than "normal." However, investigators at The Medical College of Wisconsin have found that a more important contributor to GER is that the waves of relaxation which normally follow the propulsion of the bolus down the esophagus to the stomach are extended in duration. This prolonged relaxation then permits fluid from the stomach to reflux into the esophagus.

Additional investigations have been underway to examine mechanisms of cell damage and destruction in the acute phase of esophagitis. Grantees at the Univeristy of North Carolina have been looking into the effect of bathing the esophagus with HCl solutions and determining the effects after controlled changes of the resting membrane potential difference (PD) have been achieved. These investigators found that some of the basic electrochemical properties of the esophageal membranes were disrupted. For example, when the resting membrane potential difference was reduced by acid bath to 0 - 20% of the resting PD, there were significant decreases in mucosal resistance, short circuit current and a complete inhibition of net sodium transport.

Extrapolating from similar studies done on frog tissues, these findings may be explained by an increase in sodium permeability of the cells and the intracellular junctions leading to an increase in water flow. Continued hydrogen ion exposure produces a loss of volume regulation of actively transporting cells and their eventual osmotic death. It is therefore, concluded that sodium transport functions are necessary for normal architecture and barrier function of the esophageal epithelium.

Functional and morphological studies have been underway at the VA medical Center at West Haven, Connecticut to further elucidate the mechanisms operating in the maintenance or lack of maintenance of LESP. Clinicians have known for some time that critically ill patients often suffer from GER and pulmonary aspiration. Using a cat shock model in which controlled blood loss was maintained, it was found that among the complications observed was that LESP is decreased and the response to the drug bethanechol (a compound stimulating smooth muscle contraction in the intestinal tract and, therefore, usually increasing LESP) is significantly impaired. When the cats were returned to a basal state, the basal tone of the LES returns as does the response to bethanechol. These data suggest that some as yet undefined parameter or set of parameters associated with this model predisposes the critically ill patient to GER and possibly aspiration.

The vagus nerve is known to have a significant impact on the basal tonus of the LES. Studies underway at the University of Texas in San Antonio have been examining the role of prostaglandins in the maintenance of the LES. Studies in which prostaglandins were antagonized by the drug indomethacin realized no change in the basal level of the LESP. These data suggest that PG may not be an important modulator of vagal activity. Additional studies at the Center for Ulcer Research and Education (CURE) at UCLA have shown that Gastrin in its 17 and 34 amino acid forms has some stimulatory effect for increasing human LESP. However, controlled studies after a peptone that meal indicated no significant increase in LESP. The authors go on to conclude G17 and G34 are not likely to be physiologic regulators of human LES.

Although the prostaglandins and the gastrins don't appear to have a significant effect on LESP, the peptide known as Vasoactive Intestinal Polypeptide (VIP) has recently been shown to influence LESP. Investigators at The University of Texas, San Antonio have used immunologic techniques to microscopically identify the presence of VIP in the intramural nerves innervating the LES. This compound was shown to be released when the vagus nerve is stimulated. If the VIP is antagonized immunologically, the response to vagal inhibition in diminished. The authors then conclude that VIP is and inhibitory peptide in the control of vagal innervation of the LES.

Another suggested mediator of neurological events has also been studied at the VA Medical Center at West Haven, Connecticut. There has been a very rapid expansion in the amount of information available concerning the role of morphine like compounds, endorphins, in the normal functioning of the central nervous system and the digestive tract hypothesizing that this class of compounds may have an impact on esophageal function since nerves secreting these compounds have been identified in the intestinal tract, studies using morphine and enkephalin were undertaken. In vivo studies showed that morphine produces a relaxation of the opossum LES thru a neural pathway. If the pathway is blocked by the drug tetrodotoxin, morphine is capable of acting directly on the LES circular muscle to produce contraction. It is possible, therefore, that this class of compounds may act directly exerting its myogenic effect.

2. Advances in Gastric Research

A significant amount of high quality work has been produced dealing with the physiology and pathology of the stomach. This section of the report will deal with the topics: regulation of acid secretion, production of mucus, and and close with an interesting methodological development. Interspersed throughout will be references to new data relating to the role of the prostaglandins to the various gastric functions.

a. Gastric acid secretion

An interesting line of investigation at the University of California at Berkley deals with the presence and role of naturally occurring microfilaments in the acid secreting cells (oxyntic) of the stomach.

Two lines of evidence have appeared to suggest that microfilaments are involved in the acid secretory process. First, microscopic observations made during the phases of the acid secretory cycle show systematic changes in the microfilament ultrastructure in the cells. Second, there is a positive relationship between the inhibition of hydrogen ion secretion and the microfilament disruption which results from treatment with Cytochalasin B. Subsequent investigations have shown that the microfilaments appear to be composed of actin (one of the muscular contractile proteins), that the filaments are highly organized, and that a biochemical fraction from hog gastric epithelial cells is rich in actin and myosin (a second muscular contractile protein).

Another interesting development is the suggestion that the ability of the oxntic cells of the stomach to recognize gastrin, a hormone which stimulates the secretion of gastric acid, takes time to develop after birth; at least in rats. Grantees at the University of Texas Health Sciences Center at Houston have found the newbron animals have no detectable gastrin binding ability and have extraordinarily high levels of circulating gastrin. During the course of the neonatal rats' development, circulating gastrin levels decline until at approximately 40 days of age when the gastrin levels are at adult levels. The relation between this decrease to day 40 and the time of weaning was also investigated. It now appears that the ingestion of solid food contributes to but is not the sole source of this gastrin reduction and gastric sensitivity to gastrin.

The importance of a balanced animal diet for the normal post-prandial release of gastrin was pointed out by another investigator at the University of Texas Health Sciences Center at Houston. Testing a number of chemically defined diets which are used for various types of experimental procedures, it was found that all rats ingesting these chemically defined diets had gastrin levels that were significantly less than the gastrin levels found in rats receiving a lab chow diet. The investigator concluded that either there is an important element missing from the diets or that there was some undefined elements in the diet which was inhibiting the release of gastrin.

Since the observations that naturally occurring prostaglandins (PGs) have a cytoprotective effect on the gastric mucosa, many investigations have been conducted in an attempt to determine the precise mechanism(s) by which this protection occurs. Investigations at CURE have recently examined the role of PGs in the secretion of bicarbonate ion, a gastric secretion which neutralizes the effect of the stomach's acid. Studies were undertaken to ascertain the role of topical and intravenously (IV) applied prostaglandins on bicarbonate ion secretion. Initial studies indicate that topically applied PGE2 stimulates bicarbonate secretion more effectively than IV PGE2, a situation which is likely due to the rapid metabolism of the PGs as they pass through various organs such as the lungs. Further studies showed that an increase in the hydrogen ion concentration bathing the gastric mucosa does not increase bicarbonate entry into the lumen of the stomach. Lastly, the PGE2 stimulated bicarbonate secretion was shown to be sensitive to the carbonic anhydrase inhibitor, acetazolamide.

Stress ulceration is a topic which throughout the years has received a good deal of attention, especially in the popular press. Two laboratories supported by NIADDK are presently working on two different aspects to this problem. Investigators at the University of Virginia School of Medicine have been interested in erosive gastritis and stress ulceration. This group has found that in alkaline reflux gastritis (the post gastrectomy syndrome) the severity of the problem is related positively with the concentration of intragastric bile acids which are forced into the pyloric end of the stomach by the small intestine. Their investigations have shown that one of the bile acids, taurodeoxycholic acid (TDC), produces a greater decrease in the membrane potential difference of the pyloric gastric cells, a greater increase in mucosal permeabilty to hydrogen ion, and in ischemic mucosa a more serious degree of gross anatomical damage than taurocholic acid. The investigators hypothesize that the greater destructive power of TDC may be due to the fact that it has a much greater lipid solubility than taurocholic acid.

Other investigators at the Montefiore Hospital and Albert Einstein School of Medicine in New York City have discovered that the premature separation of a mother rat from her pups will predispose the pups to a high incidence of restraint-induced gastric erosions later in life. The increased susceptibility is induced by the early loss of maternal milk and is expressed as a disturbance of body temperature at the time of restraint. In isolating the factors involved, the group discovered that a consequence of the body temperature disturbance is an increase in gastric hydrogen ion secretion during the restraint period. It is also of note that this susceptibility is not limited to the F1 progeny. If one takes the prematurely separated female rats and mates them with stock males, the progeny of this pairing produces offspring which also have and increased tendency to get restraint induced gastric ulcers.

b. Gastric Mucus Production

The importance of mucus in the protection of the stomach against autodigestion of its own secretions has been suggested for many years. With the recent advances in technology significant strides have been made in identifying more precisely what the mucus is and what causes it to be secreted. Two series of studies relating to the composition and production of gastric mucus will be described here.

One group of investigators at the New York Medical College has been interested in the biochemical composition of the lipid portions of the gastric mucus. They have found that in the pyloric region of the stomach the mucus contains much higher concentrations of glyeroglucolipds than mucus from any other portion of the stomach. They have proposed that these lipid bind to the gastric mucosa tightly in the region of the pylorus and thereby serve as a protective molecular layer for the mucosa. This hypothesis is supported by the observation that the ulcerogenic activity of lysolecithins and other lipid solubilizing agents in the duodenum are associated with their ability to deplete the gastric mucosa barrier of the glycerglucolipids, thereby disrupting the defensive layer. Such studies may lead to the production of agents which might be administered to patients and which would have the same protective properties.

The role of prostaglandins in mucus production has been investigated by scientists at CURE in Los Angeles. These investigators found that there was a significant difference in the non-stimulated mucosal layer thickness in the rat, guinea pig, dog and human. The species differences raise interesting possibilities in terms of susceptibility of the different species to the ulceration process under standard conditions. If there is a difference in ulcerogenic susceptibility, is there a correlation with the thickness of the mucus? If so, are there any chemical factors within the different types of mucus which enhance the protective effect even though the layer is thinner? Such possibilities are worthy of investigation.

Several factors were found which could alter the thickness of the mucus layer. Causing distension of the stomach by pyloric ligation caused an increase in mucus thickness that was directly proportional to the degree of distension. In addition mucus secretion could also be produced by the topical application of PGE2 in a dose dependent fashion. Another agent, carbenoxolone (an aldosterone like steroid that promotes gastric ulcer healing), was also found to increase the gel mucus thickness. It appears as a result of these investigations that the secretion of mucus is a dynamic process undergoing change based on the physiological and pharmacological state of the animal. It would be interesting to know whether the ability to secrete mucus varies over time.

c. Measurement of Intravesicular pH

It would be helpful to the understanding of the acid secretory process to be able to identify changes in the pH gradient within individual cells. Previous methods have the the drawbacks of having to using high concentrations of probe, measuring the volume of the cell in question and using fluoresence quenching as the indicator. Investigators at the University of California at Berkley have developed a novel approach to measure the intravesicular pH of cells using fluorescent probes. Basically, the method uses the pH dependent shift of the emission maximum of 2 fluorescent amines, quinine and acridine. These two probes distribute themselves according to the existing pH gradients. Then by comparing the fluoresence in acidic and basic environments, it is possible to determine the internal pH of the cell. These results were published in BBA (601: 152, 1980).

3. Advances in Colonic Research

Research has continued on a number of fronts to further understand the mechanisms operating in the colon under normal and pathologcal conditions. This discussion will be limited to a brief description of activities being conducted in two particular areas. The first dealing with inflammatory bowel disease (IBD) and the second dealing with a related problem; that of post antibiotic bacterial colonization of the colon by CLOSTRIDIUM DIFFICLE.

a. IBD

Most of the work dealing with IBD, colitis, ulcerative colitis and, Crohn's Disease, has centered around the functioning of the immune system in these patients. One test which has seen some success in explaining some of the problems associated with IBD is the mixed lymphocyte culture response (MLC). It is believed that a healthy immune system is constantly "alert" to the presence of transformed cells which may, for example, be pre-malignant or newly formed malignant cells. With a depression of the immune system, it is held that the resistance to such abnormal cells is depressed and the subject is at high risk for developing a neoplasm.

One test which is used to assess immune function is called the mixed lymphocyte culture response. Researchers at Harvard University have found that 53% of the patients with IBD who were tested with the MLC were found to have had a significant depression in that lymphocyte response. These data, which were controlled for treatment regimens, suggest that the immune system in the tested patients was compromised.

Another group of investigators at the Howard Hughes Medical Research Institute and Washington University in St. Louis, has been looking into the changes which occur in antibody production in patients with IBD and Chron's Disease. These investigators have found two distinctly different patterns of secretion of three antibodies: IgG, IgM and IgA. In normal individuals and patients with ulcerative colitis, some of the cells which produce the above antibodies, the intestinal mononuclear cells, were found to have a moderately increased spontaneous secretion of IgG and IgM and a marked increase in the spontaneous secretion of IgA. In Crohn's tissue Intestinal mononuclear cells exhibited IgG, IgM and IgA secretory patterns which were similar to normal peripheral blood mononuclear cells with low spontaneous secretion. The results suggest that further investigations should be undertaken with emphasis on cell regulatory mechanisms to determine the antigens toward which the antibodies are directed.

b. CLOSTRIDIUM DIFFICLE Complications of Antibiotic Therapy

Antibiotics are widely known to the medical profession to generate a form of colitis characterized by watery diarrhea. One of the organisms which has been commonly implicated in this disease is CLOSTRIDIUM DIFFICLE. This organism colonizes the bowel after the endogenous bacteria have been destroyed by the antibiotic therapy. The toxin produced by C. DIFFICLE produces the untoward results of diarrhea and dehydration.

Investigators at Harvard University have found a very strong correlation between the presence of the bacterial toxin in the stools in patients suffering relapses of IBD. There results suggested that the association of the toxin with the disease increases substantially with the severity of the attack. For example, in mild attacks of IBD the toxin was not found in any of 25 patients. But in 15 patients who had severe flares, the toxin was detected in 9 patients. Therapy with the anitbiotic vancomycin was effective in 5 of 8 patients tested. The authors conclude that C. DIFFICLE infection may accompany symptomatic relapse of chronic IBD even in the absence of prior antibiotic exposure.

Investigators at the University of Michigan have looked at a number of problems with C. DIFFICLE. Not only does the treatment with an antibiotic render the patient vulnerable to colonization with diarrhea, other stress such as surgery to the alimentary tract, and treatment with immunosuppressive drugs has also been shown to increase the likelihood of such an attack. This group has found the anticancer drug, methotrexate, a known immunosuppressor, also predisposes the patient to C. DIFFICLE infection. The investigators conclude that anticancer drugs should also be added to the list of potential agents which may cause C. DIFFICLE colitis.

In investigating some of the epidemiological characteristics of C. DIFFICLE this group also found that the organism is extraordianrily hearty. When areas of a floor were innoculated with this organism, it was still possible to detect it with culture methods 5 months later. This observation coupled with the findings that the organism can be cultured from the walls of hospital rooms of patients with C. DIFFICLE infection and from the hands of the staff who are caring for those patients, led the group to further conclude that the organism can evidently be spread with ease and that precautions should be taken 1. to prevent the exposure of patients who are receiving antibiotic therapy to other patients who have identified C. DIFFICLE infections; and 2. to ensure that infected animals in animal colonies are quarantined to prevent the spread of the disease throughout the whole colony.

LIVER AND BILIARY TRACT DISEASES

1980 - 1981

Overview

The program area supports research concerning the broad area of liver and biliary tract disease, including basic physiology, metabolism and morphology. Basic studies on collagen metabolism as it relates to liver fibrosis and cirrhosis; Wilson's disease, primary biliary cirrhosis, hepatic coma and portal hypertension are all areas of investigation. Bile pigment metabolism and its relationship to jaundice, Gilberts', Dubin-Johnson and Crigler-Najjar syndrome and hepatic porphyria are another group of metabolic diseases under study. Also in the program are studies on liver regeneration, liver transplantation and liver support devices as well as the morphologic liver abnormalities of Reye's syndrome, fatty liver and alcoholic hyaline. Diseases of the biliary tract such as cholesterol and pigment gallstone disease, cholestasis and biliary tract diseases of the newborn such as biliary atresia are also part of the program.

Highlights of research activities of grant-supported investigators will be found in the first section of the following report. Results of the clinical trial on cholesterol gallstone dissolution and a description of two workshops will be found in the remaining sections of the report.

I. Research and Center Grant Activity

1. Liver Cell Heterogeneity

We have come almost full circle in the nearly 30 years since Rappoport defined the liver structure in terms of functional microvascular units (acini) rather than morphologic units (lobules). Recent innovative and technically tedious research reviewed by Gumucio and Miller (Gastro 1981: 80, 393-403) makes a very convincing case that one liver cell is not like the next liver cell and that this heterogeneity of function is dependent at least in part on their location in the acini. The inlet for the very small branches carrying portal and arterial blood enter into an area of liver cells termed zone 1, pass through a rather ill-demarcated zone 2 and the blood exits via the terminal hepatic vein at the end of zone 3. As the blood enters in zone 1, it is sequentially distributed on each side of the axis to about 20 liver cells before emptying into the terminal hepatic venules of zone 3. In zone 1, the sinusoids are highly anastomosed whereas those of zone 3 are straight. Differences in metabolism, cell size, drug metabolism, liver damage and bile formation have been demonstrated between these zones. These differences have broad implications for understanding liver disease at the clinical level.

Metabolism: After eating, glycogen is stored initially in zone 1 and later gets to the central (zone 3) area. In break-down of glycogen just the opposite is seen - glycogen disappears first from zone 3. When tissue from these areas are microdissected and analyzed by extremely sensitive methods, the enzymes necessary for glycogen synthesis are found to be located in zone 1 (inlet area) whereas enzymes required for glycogen breakdown are located predominately in zone 3 (outlet area), a good correlation of structure with function. Blood with high loads of nutrients from gut absorption enters the liver in zone 1 and glycogen is readily made as a storage form of energy. These zonal differences are not found for protein metabolism and data on the localization of lipid metabolism is still insufficient.

Bile Formation: Another grantee (A. C. Jones, U. Calif., Am. J. Physiol. 238: G 233-237 (1980) showed directly by autoradiography that the first 6-9 liver cells remove almost 60% of bile acids from the sinusoidal blood entering at zone 1. He accomplished this direct measurement where others had been unsuccessful by using an iodinated poorly soluble bile acid analog, ^{125}I-cholylglycylhistamine. This lobular concentration gradient for bile salts need not be due to any intrinsic properties of the liver cells in zone 1 but may be expected for substances which undergo an efficient extraction from sinusoidal blood. Therefore, under physiological conditions, the cells of acinar zone 1 are primarily involved in secretion of the bile-salt dependent fraction of bile. On the other hand, cells of zone 3 may be the ones contributing to the bile-salt independent flow because damage to zone 3 by carbon-tetrachloride, is associated with decreases in bile flow but no interference with zone 1 bile acid dependent flow.

The recent microdissection of acinar zones, the development of very sensitive methods of analysis and the separation by the density or volume of the cells are all opening up new avenues for research in this exciting area.

2. Pigment Gallstones

About 25% of the patients with gallstones in the United States may have a little studied and poorly understood type of gallstone, the pigment stone. Only a few investigators in the United States are studying this type of disorder so that an international workshop was held to bring together investigators from various countries. There are two major types of pigment stones: the calcium bilirubinate or brown stone forms mainly in the bile ducts and may be caused by bile stasis and bacterial infection. It is soft, pliable and composed of bilirubin, calcium and cholesterol. The second type is the pure pigment stone or black stone which forms only in the gallbladder, it is hard and brittle and in some cases is associated with overproduction of bilirubin such as occurs in spherocystosis. These stones are composed of bilirubin, an unextractable black residue and a very small amount of cholesterol. Soloway, Univ. of Pennsylvania, in a study, found that pigment stones seem to occur in an older population (older than 70 yrs.) than do cholesterol stones. All stones found in a single gallbladder are of the same type. A very interesting observation is the geographical differences in the types of stones obtained at surgery. For example, in the Bolivian population, no pigment stones were found, only cholesterol stones, which may be because the population dies at too early an age for pigment stones to develop. Calcium bilirubinate stones (brown stones) have a very low incidence in Europe and the U.S. but are frequent in East Asia. In Japan, for example, about 50% of all gallstones in the prewar period were of this type but this proportion has been steadily decreasing probably as a result of dietary changes and urbanization. The black stone is probably the most difficult to characterize chemically since much of the material in the stones is virtually insoluble in all solvents. A polymer chemist working with a former grantee, Donald Ostrow, (now VA-supported), have data indicating that this pigment is a highly crosslinked polymer. Bilirubin is probably polymerized in this network via its vinyl groups, leaving its carboxyl groups free. This would be compatable with the much greater degree of swelling of this polymer seen at alkaline pH.

The research on pigment gallstones is following the lead of cholesterol gallstones where a condition of supersaturation of the bile with cholesterol could be correlated with cholesterol gallstone formation. Thus, Carey from Boston has constructed phase diagrams to produce metastable and supersaturation zones for unconjugated bilirubin as functions of acidity, calcium ion concentration, total lipid concentration and the bile salt-lecithin-cholesterol ratio. When reliable analytical data from the literature are plotted on this phase diagram, the results show that "pigment" biles are markedly supersaturated with unconjugated bilirubin whereas biles from animals without stones and from patients with cholesterol stones are unsaturated with bilirubin.

Medical dissolution of bilirubinate stones retained in the common bile duct after surgery is a therapeutic goal. Although mono-octanoin has proved effective in this respect for cholesterol stones, it has little effect on pigment stones. An investigator in Frankfurt has tried a novel approach in vitro using human pigment stones. The stones are put into alternating solutions of the mono-octanoin to dissolve the cholesterol, then EDTA buffered with carnosine to chelate the calcium pigments and get them out from their cross linking with organic substances. Dissolution occurred in 40-60 hours. In vivo, these investigators claim therapeutic success with alternating treatment in 16 of 24 patients.

3. Liver Cell Death

There are many agents, viral, chemical, and bacteriological, which produce liver cell necrosis or death. The mechanisms may be different by which the process of irreversible damage is initiated in each of these cases. However, Dr. John Farber, a grantee at Temple Univ. is working on the hypothesis that a common mechanism may be involved in several or all. He is looking at the actions of three different hepatotoxins: galactosamine, phalloidin and alcohol. Toxic liver necrosis generally involves damage to the plasma membrane which results in an influx of lethal amounts of calcium ions. Using primary cultures of adult rat liver cells, two of the hepatotoxins, galactosamine and phalloidin, act on the plasma membrane to cause reversible cell injury in the absence of extracellular calcium and lethal injury in its presence. In both cases, there is probably disruption of the permeability barrier function of the plasma membrane, but the mechanism of damage is different for each toxin. Phalloidin, a toxic compound from the poisonous mushroom, polymerizes actin molecules that are either associated with or embedded into the plasma membrane. If one gives an agent like Cytochalasin B which reverses the effect on actin polymerization, one can prevent phalloidin from causing irreversible liver cell damage in the presence of calcium. The other toxin, galactosamine, induces a deficiency of uridine nucleotides which results in altered plasma membrane function. Giving uridine or uridine-containing compounds can prevent the cell death which accompanies the addition of calcium to cells previously treated with galactosamine. A widely used toxin, alcohol, seems to potentiate the liver injury of both of these poisons. That is, doses of phalloidin or galactosamine that cause only minimal cell death, combined with a dose of alcohol that causes no cell death, will cause 50% of the cells to die (in the presence of calcium) when combined.

This data may be very relevant to the problem of the sudden appearance of necrosis with alcoholic hepatitis after years of alcohol ingestion. Random exposure of the liver to membrane-active toxins may ordinarily be well tolerated. Examples of such toxins may include products of intestinal bacteria, ingested xenobiotics, viruses and drugs. However, in the presence of alcohol, such agents may overwhelm the homeostatic mechanisms of the cell by permitting an influx of lethal amounts of calcium.

4. Other vignettes of interesting research advances this past year are the following:

- Time lapse photography of cultured liver cells that maintain an intact tight junction show propulsive, active contraction of their bile caniliculi. This action appears to be mediated by actin filaments around the caniliculi because cholestasis occurs when the filaments are inhibited. This active contraction, never seen before, may be responsible along with hydrostatic and osmotic forces for the flow of bile. (M. J. Phillips, Toronto)

- Zellweger's syndrome, characterized only in 1964, is a very rare disease showing severe growth and mental retardation, renal cortical cysts, and liver dysfunction with abnormal mitochondrial function. Using patients with this disease, it was shown that unusual bile acids form and accumulate because cleavage of the side chain of cholesterol to form bile acids cannot occur because of the mitochondrial dysfunction. (R. U. Hanson, U. Minn.)

- A non-absorbable form of sucrose (sucrose polyester), with the consistency of fat, when incorporated into the diet of patients maintained on a 1000 cal/day diet, was able to lower plasma cholesterol more than the weight loss - low cholesterol diet alone. (S. Grundy, U. Calif., San Diego)

- For the past decade, hepatologists have believed that the histologic appearance of "bridging necrosis" in a liver biopsy identified patients with acute viral hepatitis who were at special risk to either have a fulminant course of the disease or to develop cirrhosis. According to a prospective clinical trial, the finding of bridging necrosis has no prognostic usefulness when the acute viral hepatitis is of less than 1 month duration. These patients usually have a benign clinical course with less than 5% developing chronic hepatitis. (Ware, A., U. Texas, Southwestern)

II. Workshops

1. An NIAMDD sponsored "International Workshop on Pigment Gallstone Disease" was held May 13-15, 1981 at the University of Pennsylvania in Philadelphia. The organizers and NIAMDD grantees were Drs. Roger Soloway and Bruce Trotman. The topics covered included the classification of pigment gallstones, epidemiologic features, radiographic assessment of pigment stones, composition of pigment gallstones, and the composition of bile in patients with pigment stone disease. About 50 people participated, including investigators from Japan, Austria, Scotland, Belgium, England, West Germany in addition to the U.S. investigators.

2. A workshop, jointly sponsored by the American Association for the Study of Liver Disease and the NIAMDD was held November 5, 1980 in Chicago. The title was "Physical and Biological Research Techniques" and the purpose was to provide intellectual stimulation for the young research trainee and fellow working in

liver research. Fifty attendees participated in this one day workshop in which six senior investigators presented such subjects as gas-chromatography-mass spectroscopy; quasielastic light scattering; electron probe analysis; receptors; membrane vesicles and radioimmunoassay.

National Cooperative Gallstone Study (NCGS)
N01-AM-3-2216; N01-AM-0-02205
Cedars-Sinai Medical Center FY80 = $1,247,479

III. The Dissolution of Cholesterol Gallstones

This eight year cooperative clinical trial of 916 patients with cholesterol gallstones has been completed, including publication of the main results which will appear in the September 1981 issue of Annals of Internal Medicine. The total cost has been $10,245,000 from June 1973 through March 1981. The contract will continue through February 1983 to accomplish 4 additional tasks: (1) complete analysis of data from sub-groups and complete 10 additional manuscripts on the study; (2) complete the Phase III recurrence study of patients in whom stones have dissolved, (3) monitor the group of former placebo patients who have now received the drug, and (4) prepare all data and patient material for turnover to NIH for repository purposes.

The study was designed to test the efficacy and safety of two doses of chenodeoxycholic (CDCA) acid on the dissolution of cholesterol gallstones. Earlier basic investigations, also supported by the NIAMDD, led to the discovery in 1970 at the Mayo Clinic by Danzinger, Schoenfield, Hofmann and Thistle that CDCA could dissolve gallstones. This was the first potential therapy providing an alternative to an operation. The NCGS was prompted primarily because of the major impact of gallstones on health care in the U.S. and because insufficient information was available on the degree of efficacy and safety of CDCA.

The randomization of treatment to the first of 916 patients at 10 hospital centers participating in the major study of the NCGS occurred in November 1976, and the last patient completed two years of follow-up in August 1980. Three-hundred and five patients received 750 mg CDCA per day, 306 received 375 mg CDCA per day, and 305 received placebo. The patients were monitored clinically and by blood tests and x-rays of the gallbladder at prescribed intervals during their entire participation.

Following are the main findings of the NCGS after two years of treatment with 750 mg CDCA per day:

1. Benefits of CDCA

    a. Complete dissolution of gallstones occurred in 14% of patients and partial dissolution in an additional 27%.

    b. Complete dissolution occurred more often in thin patients or those with small gallstones or with high normal serum cholesterol levels.

    c. Although partial and progressive dissolution were demonstrated, the NCGS provided no information on the efficacy and safety of treatment with CDCA beyond two years.

2. Effect of CDCA on Symptoms or the Need for Operation

The frequency of abdominal pain caused by gallstones or the need for cholecystectomy during the two years of treatment were neither reduced nor increased by CDCA.

3. Side Effects of CDCA

a. Loose bowel movements occurred in 41% of patients, but this diarrhea was mild.

b. Clinically significant injury to the liver occurred in 3% of patients, but the biochemical abnormalities were reversible in each case.

c. A mild (10%) elevation of the average serum cholesterol occurred which could impose a slightly increased risk of developing heart disease in those patients with an elevation.

The NCGS concluded that chenodeoxycholic acid, 750 mg per day, has a role in the treatment of appropriately selected patients with cholesterol gallstones. Treatment should be predicated on an understanding of the potential benefits and risks of the drug compared to those of operation or observation. The FDA now will evaluate the results of the NCGS and the pending NDA applications from pharmaceutical companies to determine whether to allow the companies to determine whether to allow the companies to market CDCA in the U.S.

ANNUAL REPORT (1980-1981)

INTESTINAL AND PANCREATIC DISEASES PROGRAM

Although a considerable amount of research and other activities, both basic and clinical, has been either reported by the grantees or initiated by the Program during the past year, only a few examples will be cited to illustrate the progress that has been made in various areas of interest to the Program.

1. Small Intestine:

(a) The intestinal brush border aminooligopeptidases are integral membrane proteins located at the luminal surface of the small intestinal cell. These digestive hydrolases are probably transmembrane proteins whose active site is available at the lumen-cell interface to ensure surface hydrolysis of oligopeptides of four to six amino acid residues which cannot be assimilated intact across the intestinal surface membrane. Despite the importance of these enzymes for protein digestion, and as in the case of other integral membrane proteins in mammalian systems, their intracellular site of synthesis, mode of intracellular translocation, and mechanism of membrane insertion are unknown. Dr. Gray et al. (RO AM 15802 and T32 AM 07056) at Stanford University School of Medicine have identified aminooligopeptidases in rat jejunum mucosal cytosol which cross-react in a sensitive radioimmunoassay for the brush border peptidases. These cytosol peptidases were purified by antibody-affinity chromatography, and their physical and chemical characteristics were compared with those of their brush border counterparts. Analysis revealed that the cytosol enzymes exist as three molecular weight isomers, having a common subunit present as either a monomer (70,000), dimer (140,000), or tetramer (280,000). Two of these cytosol species are smaller by 40,000 daltons than the comparable brush border aminopeptidases. Binding of the cytosol peptidases to concanavalin A-Sepharose was less complete than that found for the related brush border enzymes. Incorporation of (^{3}H)-leucine in vivo into cytosol aminopeptidases was maximal at 20 min but was not achieved in the brush border membrane aminopeptidase until 1-3 h. These cytosol aminopeptidases have the appropriate characteristics to serve as precursors of the surface membrane peptidases at some stage of the intracellular assembly process.

(b) Microvilli are fingerlike plasma membrane specializations found on the free surfaces of most cells. The microvillus cytoskeleton is composed, in part, of a core bundle of actin microfilaments which inserts end-on into an electron-dense cap at the microvillus tip. The remainder of the microvillus cytoskeleton includes cross-links which appear to link the microfilaments into a bundle and periodic lateral arms (cross-filaments or cross-bridges) which connect the bundle laterally to the microvillus membrane. The absorptive surface of some epithelial cells, such as those of the intestine, are covered with hexagonally packed microvilli organized into a structure termed the brush border. Dr. Burgess et al. (R01 AM 20021) at Dartmouth College, Hanover, N.H. have examined the effects of ATP and deoxycholate (DOC) on the cytoskeletal organization of Triton-demembranated microvilli isolated from chicken intestine brush borders. Isolated microvilli are composed of a core of tightly bundled microfilaments from which arms project laterally to the plasma membrane with a 33-nm periodicity. These lateral arms spiral around the core microfilaments as a helix with a 25° pitch. Demembranated microvilli consist of four polypeptides with mol wt of 110,000, 95,000, 68,000, and 42,000, present in molar ratios of 1.1:1.6:1.3:10.0. After addition of 50 uM ATP and 0.1 mM Mg^{++}, the cytoskeletons are organized as a tight bundle of microfilaments from which lateral arms are missing. In these ATP-treated cytoskeletons, the 110-kdalton polypeptide is reduced in amount and the 95,000, 68,000, and 42,000 polypeptides are present in a 1.3:1.2:10.0 ratio. In contrast, after incubation with 0.5% DOC, the core microfilaments are no longer tightly bundled, yet the lateral arms remain attached with a distinct 33-nm periodicity. These DOC-treated cytoskeletons are depleted of the 95,000 and 68,000 polypeptides and are composed of the 110,000 and 42,000 polypeptides in a 2:10 molar ratio. These results suggest that the microfilaments are associated into a core bundle by the 95- and 68-kdalton polypeptides and from this core bundle project the lateral arms composed of the 110-kdalton polypeptide.

(c) The study of diseases of the intestinal epithelium (mucosa) has always been limited by the need for mucosal biopsies to examine the histology and the enzymes characteristic of an intact, mature mucosa. Dr. Luk et al. (K08 AM 00774) at Johns Hopkins University School of Medicine have recently reported that the activity of diamine oxidase (DAO; histaminase), an enzyme found in high concentrations in the intestinal mucosa of humans and other mammalian species, may be the first known circulating marker for monitoring mucosal maturation and integrity. Specifically, they investigated whether plasma and mucosal levels of DAO activity reflect both the maturational status of the mucosa during its developmen in the newborn rat and the degree of mucosal damage during its injury in the adult rat. Litter mates were reared under identical conditions and killed at different ages from day 0 to day 40 after birth. DAO in the small intestine was low at birth, increased gradually with age, reached a peak at 22 days, and then remained at normal adult levels, similar to the development patterns of maltase and sucrase. Plasma DAO rose in parallel with intestinal levels, reached a peak at 24 days, and then remained at normal adult levels. DAO activity in 15 non-intestinal tissues was less than 5% of ileal mucosal activity, and no nonintestinal activities showed increase with age. Adult rat intestinal loops were perfused with hyperosmolar sodium sulfate solutions to produce selective damage to villus mucosa. With increasing mucosal damage, there was a progressive decrease in the enzyme activities studied; first, lactase levels fell, then maltase and sucrase, and finally mucosal and plasma DAO activity levels fell. The decrease in plasma DAO reflected the degree of mucosal damage. DAO activity is thus unique among intestinal mucosal enzymes studied to date in that circulating levels can serve as a marker of mucosal maturation and integrity.

(d) During the intestinal absorption of cholesterol, 70-90% of the latter undergoes esterification. The efficiency of this esterification is a regulatory factor in cholesterol absorption. A cholesterol esterifying enzyme, cholesterol esterase, active in crude extracts of intestinal mucosa from rat and other species and associated with isolated rat intestinal cells, can account for cholesterol flux from the intestine. However, there are conflicting reports concerning the localization and origin of this enzyme. Using an immunocytochemical approach which employed monospecific rabbit antisera to rat pancreas cholesterol esterase, Dr. Gallo et al. (R01 AM 26346) at the George Washington University School of Medicine and Health Sciences determined the localization of this pancreatic enzyme within the wall of rat small intestine. The enzyme was found to be uniformly distributed within the absorptive cells, but was notably absent from the microvillar membrane. Enzyme was also seen within the lamina propria and submucosa. In contrast, enzyme was absent from sections of proximal intestine surgically deprived of pancreatic juice for 72 hours. Furthermore, the enzyme concentration in sections of normal intestine decreased with increasing distance from the pancreatic duct. These observations support the concept that "intestinal" cholesterol esterase is of pancreatic origin. This enzyme is localized within the cells as opposed to the absorptive surface.

(e) Long-chain fatty acids are absorbed by a process that requires an obligatory intracellular esterification. Different regions of the small intestine possess different capacities for fatty acid esterification. However, the differences in the esterification capacity along the villus-crypt structure have not been evaluated. It is well known that intestinal epithelial cells mature while migrating from the base of the crypt to the tip of the villi. This is reflected in differentiation of various enzyme activities along the villus-crypt structure. Brush border disaccharidases and alkaline phosphatase activities are high in the villus cells while absent in the crypts. In contrast, thymidine kinase activity is predominantly concentrated in crypt cells. While varying enzyme distributions along the villus-crypt unit have been interpreted as a "functional differentiation," little information is actually available concerning the differentiation of absorptive function along the villus-crypt units. Dr. Koldovsky et al. (R01 AM 27624) at the University of Arizona have recently measured the fatty acid esterification capacity (i.e., incorporation of fatty acid into triglyceride) and lipid esterifying enzyme activities (viz., acyl CoA synthetase and acyl CoA monoglyceride acyl transferase) along the height of the villus-crypt units in rat jejunum. Their data show that (a) fatty acid esterification capacity is apically concentrated on the villus-crypt units, and (b) fatty acid esterifying enzymes have a similar distribution pattern, implying that the apical distribution of esterification capacity is a property of the villus-crypt unit.

(f) Heat-stable enterotoxin, elaborated by some enteric strains of Escherichia coli, has been implicated as a causative agent of diarrhea in both infants and adults. This toxin is a small polypeptide with reported molecular weights ranging from 1972 to 5100 depending on the bacterial strain. Although the capacity of heat-stable enterotoxin-producing bacteria and/or culture filtrates of these bacteria to increase intestinal fluid secretion has been well documented, there was, until recently, little information about the probable mechanism by which the toxin elicits this effect. In contrast to heat-labile E. coli enterotoxin and cholera toxin, the heat-stable toxin-stimulated fluid secretion is associated with an increase of cyclic GMP but not of cyclic AMP concentration. Furthermore, this increase in cyclic GMP concentration is due to an activation of guanylate cyclase rather than to an inhibition of cyclic GMP phosphodiesterase. Drs. Field and Smith (R01 AM 21345 and F32 AM 05973) at the University of Chicago have recently examined the tissue specificity of its action and the relation of its action to those of the 8-bromo analogues of cyclic GMP and cyclic AMP. Heat-stable enterotoxin stimulated guanylate cyclase activity and increased cyclic GMP concentration throughout the small and large intestine. It increased transepithelial electric potential difference and short-circuit current in the jejunum, ileum, and caecum, but not in the duodenum or distal colon. This pattern of electrical responses was mimicked by 8-bromo-cyclic GMP. However, 8-bromo-cyclic AMP produced an electrical response in all intestinal segments. The enterotoxin failed to stimulate guanylate cyclase in liver, lung, pancreas, or gastric antral mucosa. In the intestines, it stimulated only the particulate and not the soluble form of the enzyme. Preincubation of the toxin with intestinal membranes did not render it capable of stimulating pancreatic guanylate cyclase. Cytosol factors did not enhance the toxin's stimulation of intestinal guanylate cyclase. This study supports the role of cyclic GMP as intracellular mediator for heat-stable enterotoxin and suggests that the toxin affects a membrane-mediated mechanism for guanylate cyclase activation that is unique to the intestines.

2. Pancreas:

(a) Increased pancreatic secretion is accepted as one of the important pathogenic mechanisms by which alcohol ingestion leads to acute pancreatitis. Alcohol is a strong stimulant for the release of hydrochloric acid by the stomach, which in turn stimulates secretin release by the duodenum. This produces an increase in pancreatic secretion which could lead to enzyme extravasation and pancreatitis. Thus, suppression of pancreatic secretion has long been accepted as standard therapy for acute pancreatitis. Nasogastric suction, atropine, and glucagon have all been recommended for this purpose. Recently, however, prospective, randomized clinical trials have failed to reveal any benefit from these treatment modalities. These studies have cast doubt on the role of pancreatic secretion in the pathogenesis of acute pancreatitis.

Dr. Cameron *et al.* (R01 AM 19646) at the Johns Hopkins Medical Institutions have recently evaluated the influence of the secretory state of the pancreas on the severity of acute pancreatitis induced in an isolated, ex vivo, perfused canine pancreas. A standard technique of free fatty acid (oleic acid) infusion was employed for the induction of pancreatitis. Secretin was used to stimulate secretion, and glucagon and atropine for suppression of secretion. Despite a 40-fold difference in hourly secretory output, the development of pancreatitis as judged by weight gain, hemoconcentration, serum amylase value, and arterial blood pressure response was similar in the stimulated, basal, and suppressed state. This study casts further doubt on the role of hypersecretion in the pathogenesis of acute pancreatitis.

(b) While studying the combined effects of cyclic nucleotides and peptide hormones on pancreatic secretion, Peikin and Gardner discovered that dibutyryl cyclic GMP (N^2,$O^{2'}$ -dibutyryl guanosine 3':5'-monophosphate) competitively and specifically inhibits the effect of pancreozymin and its analogs on both enzyme secretion and calcium efflux from isolated guinea pig acini. The authors inferred that the inhibition by the nucleotide takes place at the membrane receptor level. Recently, Christophe *et al.* (R01 AM 17010) at the Free University of Brussels (Belgium) have examined the effects of butyryl nucleotides on pancreozymin stimulation of amylase released from rat pancreatic fragments. Moreover, they examined directly the interaction of butyryl nucleotides on ^{125}I-pancreozymin binding and adenylate cyclase activation in rat pancreatic plasma membranes. Their findings definitely support the conclusion that butyryl derivatives of cyclic GMP inhibit the pancreatic action of pancreozymin and related peptides by virtue of their ability to interact with pancreozymin receptors. The presence of a butyryl side-chain on the purine base was essential for inhibiting hormone binding and the resulting activation of adenylate cyclase. In addition, the second butyryl side-chain on the ribose moiety allowed maximum efficacy. Since structural similarities between pancreozymin-like peptides and butyryl derivatives of cyclic GMP might be responsible for this phenomenon, they studied the interaction of these nucleotides with other pancreozymin "receptors": the antibodies directed against gastrin-pancreozymin-like peptides. They found that the nucleotides were recognized by these antibodies with a relative affinity broadly comparable to that observed with pancreozymin receptors in the rat pancreas.

3. Gastrointestinal Hormones:

(a) Cholecystokinin (CCK) is the parent molecule of a family of polypeptide hormones, some of which are secreted into the circulation from the small intestine after food ingestion and stimulate both exocrine pancreatic secretion and gallbladder contraction. Employing antibodies to various cholecystokinins, radioimmunoassay studies have identified CCK-like molecules in extracts of the central and peripheral nervous systems. In addition, CCK-like molecules have been identified in brain neurons with immunocytochemical techniques.

The biological significance of CCK in the brain is unknown. One possible neural role for this hormone is mediation of satiety. After food is introduced into the intestine, satiety is produced in various species of animals and this effect can be mimicked by systemic injections of CCK and its analogues into rats, rabbits, monkeys, and man. These hormones are also effective when injected into both the hypothalamus of rats and the cerebral ventricle of sheep. Other studies, however, suggest a peripheral rather than central action of CCK on satiety.

Straus and Yalow, employing antibodies reacting to CCK_4, reported that brain CCK levels were markedly reduced in the cerebral cortex of both genetically obese mice with the ob/ob genotype and normal mice after a 2-5 day total fast. Their studies suggest, therefore, that CCK may have a role in appetite control and that CCK levels in the brain are altered in various metabolic states. In contrast, however, Schneider et al., employing an antibody to desulfated CCK_8, did not detect changes in brain CCK levels under similar conditions.

Dr. Williams et al. (R01 AM 26422) at the Mount Zion Hospital and Medical Center, San Francisco, CA, have recently demonstrated that biologically active CCK_{33} with high specific activity can be prepared by conjugating the hormone to iodine 125 labelled Bolton-Hunter reagent. They have also shown that this ligand binds to specific receptors in both rat brain and pancreas. The highest concentration of brain CCK receptors in the rat was in the olfactory bulb, and then in the cerebral cortex, caudate, hippocampus, and hypothalamus. Lower concentrations of receptors were seen in the cerebellum. In light of the possible alterations of CCK receptors during various metabolic states in the mouse, they undertook a study to determine whether CCK receptors in mouse brain were influenced by fasting. Specifically, cholecystokinin receptors in various regions of the brain were measured in fed and 42-hour fasted mice. Fasting significantly increased cholecystokinin binding to its receptors in the olfactory bulb (46%) and hypothalamus (42%), but not in other brain regions including the cerebral cortex. Analysis of the data revealed that this increase was due to an increase in the number of receptor sites. In contrast, insulin binding to its receptors was not altered by fasting. In several species, the hypothalamus has been shown to regulate appetite. This increase in hypothalamic receptors upon fasting, therefore, supports the concept that cholecystokinin may regulate satiety via its interaction with this brain region.

4. Salivary Glands:

(a) The submandibular glands of the rat and mouse are similar in structure. However, a pronounced sexual dimorphism of the gland is seen only in the mouse, but not in the rat. Several biologically active polypeptides have been localized in considerable quantities in the granular convoluted tubule (GCT) segment of mouse submandibular gland. Some of these substances have also been detected in far lesser amounts in the submandibular glands of rats. Dr. Barka *et al*. (R01 AM 19753) at Mount Sinai School of Medicine of the City University of New York have recently provided evidence for the presence in rat submandibular gland of a factor which immunologically cross-reacts with antiserum against mouse epidermal growth factor (EGF). By using this antiserum, only the GCT cells revealed immunochemical staining in rat submandibular glands. There was no regular sexual difference in the frequency or size of immunoreactive cells. Extracts of gland contained an antigen which showed a complete cross-reactivity with mouse EGF in radioimmunoassays. The relative amounts of EGF, determined by a heterologous radioimmunoassay, were not significantly different in the glands of rats of the two sexes. Administration of testosterone caused an increase, in both sexes, in the number of GCT cells stained for EGF and in the amount of EGF in the gland. There was no significant sexual difference in these two parameters after androgen treatment.

NUTRITION PROGRAM, NIADDK
Annual Report, FY 81

The Nutrition Program of NIADDK, with a total FY81 budget of 15.15 million dollars, has continued to support extramural research and training in basic and clinical nutrition, especially in those areas where additional emphasis is needed to achieve the goal of improved health and longevity. Much of the research supported might be described as "basic" in nature, directed toward the development of new knowledge about the role of nutrients in human physiology and biochemistry. Generally, such research has been nutrient-centered rather than organ-centered or focused on a specific disease. Discoveries from basic research have advanced our understanding of the function of nutrients in the whole body and have found wide application in clinical medicine. An example is the discovery that dietary Vitamin D must be converted to a modified form to be active, and that kidney tissue is required for one step in the conversion. This has led to impressive new clinical applications, especially in bone and kidney diseases.

The widespread prevalence of nutrition-related diseases or conditions such as artherosclerosis, diabetes, obesity, osteoporosis, alcoholism, and anemia in certain segments of the population is evidence of the need for a better understanding of the nutritional aspects of these clinical problems. The greatest promise for solutions to these problems lies in fundamental studies, such as those on absorption, metabolism, mechanism of action of nutritional factors and the role of diet as a metabolic regulator. Recent technological advances, including mass spectrometry, high pressure liquid chromotography, and radioimmunoassay, should permit rapid progress in basic nutrient-centered biomedical studies.

The Nutrition Program has continued to stress the following research areas: basic studies on the metabolic role of protein, amino acids, vitamins, minerals, fatty acids, dietary energy sources and dietary fiber; research on obesity with the aim of prevention and control; nutritional status assessment and supportive nutrition of patients; human nutritional requirements and factors influencing them; interaction of nutrients and drugs; and the role of nutrition in infection and immune competence.

Nutrition also constituted a significant component of research supported by other NIADDK programs, especially Digestive Diseases, Diabetes, Metabolism, Hematology, Endocrinology and Liver Diseases Program.

Training

NIADDK continues to have a primary responsibility for training in nutrition through fellowships and training grants than other Institutes. During FY81, the Nutrition Program administered 15 active fellowships and nine training grants in nutrition. However, limited funds available for competing awards will permit funding of only three fellowships and a renewal of two training grants in clinical nutrition at a considerably reduced levels. Two new training grant applications have been approved with good priority but are being held until FY81 for possible funding. Total training funds in nutrition amounted to 1.031 million dollars. In addition, five career awards were funded in FY81.

Clinical Nutrition Research Units (CNRU)

The Nutrition Program, NIADDK, supports five of the seven CNRU's; the other two are funded by NCI. These core grants in clinical nutrition research represent a new and innovative approach to provide a focus for clinical nutrition research and related activities in biomedical institutions. These awards are expected to stimulate progress in multidisciplinary research in clinical nutrition, strengthen training environments, enhance patient care and generate nutrition information for the public.

The first annual meeting of CNRU directors was held December 3, 1980, to review progress and problems and to provide a forum of information exchange among directors and staff. A summary of the activities which have taken place as a result of the CNRU awards is being prepared for publication in The American Journal of Clinical Nutrition. Also, the first four CNRU's funded in September 1979, are being site-visited for purposes of early evaluation of this new mechanism. It appears very clear that the CNRU's have already had a considerable beneficial effect on nutrition activities at these institutions.

The following CNRU's are supported by NIADDK:

1. University of Chicago - Dr. Irwin Rosenberg, Director

The CNRU research base includes ongoing research on diabetes, arterosalerosis, urolithiasis, cancer, lipoprotein, stable isotopes, calcium metabolism, and vitamin absorption and metabolism. Together with the resources of the biological sciences faculty and nutrition support service, this CNRU provides nutritional assessment, clinical intervention, laboratory assays, and support services to foster clinical nutrition investigations.

2. Vanderbilt University - Dr. Harry L. Greene, Director

Research serviced by core laboratories includes studies on composition of defined formula diets and total perenteral nutrition mixtures; nutritional factors in hepatic encephalopathy; micronutrient metabolism in patients with essential fatty acid deficiency; nutrient supplementation, cancer chemotherapy and micronutrient metabolism; chronic dialysis on trace mineral hemostasis; nutritional assessment and nutritional support for presurgical patients. This CNRU has an ongoing training grant in nutrition and gastroenterology. A first-year pilot study dealt with identification and treatment of undernutrition in hospital patients. Vanderbilt's public information office will increase public awareness in nutrition as a part of the CNRU.

3. University of Wisconsin - Dr. Alfred E. Harper and
   Dr. Dr. Earl Shrago, Co-Directors

The CNRU is expected to provide a significant impetus to research and education in clinical nutrition as well as lead to the general improvement in clinical management and nutrition support of patients. The clinical research components include studies on nutrition and geriatrics, preventive cardiology, anorexias, lipid disorders, cancer and dialysis.

4. Medical College of Georgia - Dr. Elaine Feldman, Director

This CNRU provides core research laboratories for lipid analysis trace metal determinations, and nutritional biochemistry. The CNRU staff function in one or more of the three nutrition related areas of clinical research, education and training, and nutrition support service. The nutrition education program in The School of Medicine is supported in conjuncion with a separately funded curriculum development grant in applied nutrition. The goal is to search for the etiology and treatment of nutrition-related disorders affecting the population of Georgia.

5. Columbia University - Dr. Robert W. Winters, Director

Activities in the CNRU focus on the pediatric patient, utilyzing the large research, education and training programs already in place. Research is directed toward providing adequate nourishment to the fetus, the premature or term infant, child or adolescent. Projects with nutrition components embrace such diverse field as cardiology, oncology, neonatology, allergy, pulmonary diseases, immunology and perinatology. The CNRU provides a clinical core, a laboratory core and a computer/biostatistic core.

U.S.-Japan Malnutrition Panel

Malnutrition continues to be one of the most serious problems in Asian countries despite the many intervention programs which have been conducted. While food availability is no longer the major problem, there are social, political and economic forces which account for serious inequities in distribution. The most common states of undernutrition are protein-energy malnutrition and iron and vitamin A deficiency. Superimposed are various disease states, particularly diarrheal infections, which cause impaired utilization and loss of nutrients and become the primary cause of death of children under five. Undernutrition also has severe and adverse effects on pregnancy, and can lead to premature and low-birth-weight babies with inadequate nutrient reserves. Iron deficiency, which is widespread, is believed to interfere with the immune response, cause lower resistance to infection and reduce functional physical capacities and possibly mental and learning processes. Vitamin A deficiency in turn causes many thousand of cases of damaged vision and total blindness in young children each year.

The research fostered under the auspices of The U.S. and Japanese Malnutrition Program is primarly targeted at those areas which will most benefit the undernourished of Asia. The emphasis areas are: (1) Influence of environmental and host factors on nutritional requirements, (2) Health significance and methods of preventing iron deficiency, (3) Interaction of nutrition, immune competence, and infection, (4) Effects of nutrition on physical and mental development, behavior, physical capability and work performance, and (5) Health consequences of different and changing dietary patterns in food habits.

The NIADDK has continued to administer The U.S. Malnutrition Panel of The U.S.-Japan Cooperative Medical Services Program. The annual joint meeting of the U.S. and Japanese Malnutrition Panels will be held in the morning of August 15, 1981, with a cosponsored workshop in the afternoon. The workshop will be on "Opportunities for Collaborative Research Relating to Nutritinal Requirements in Asian Countries."

Research by Contract

NIADDK is renewing for one year its contract (N01 AM 0-2204) with the Food and Nutrition Board, National Academy of Sciences at a cost of $100,710. This contract is entitled "Preparatin of the Tenth Edition of Recommended Dietary Allowances and Related Studies." In addition to the preparation of the tenth edition of the RDAs, guidelines for use of these RDAs are to be prepared for publication. A component of the previous contract, was deleted. This dealt with the development of dietary guidelines for specific population sub-groups based on an interpretation and translation of research data relating to diet and health status.

A Committee on Dietary Allowances and a Sub-committee on Uses of the RDA have been formed and have held several working meetings using the committee study approach. Two workshops have been held, one on the variability in human nutrient requirements, and a second addressing issues concerning the uses of the RDAs.

Trends in Extramural Research

1. Obesity

Despite the emphasis given to obesity research during the past seven years, many of the physiological consequences of obesity are yet poorly defined, little appreciated, and incompletely understood. Recent studies indicated that obesity can be environmentally induced by luxus-consumption during early childhood leading to an increase in number of adiposites. Additional work on diet modification so as to avoid the increase in adipose tissue cellularity and presumably the propensity toward obesity is underway in several laboratories. Despite this, it is likely that a significant obese population will persist for years to come. It is important, therefore, to try to unravel the web of interacting consequences associated with becoming obese and maintaining the obese state. The obese individual represents an extreme in weight, bulky, energy and fat stores, peripheral insulation and lack of agility, the obese are also more likely to have hypertension, late-onset diabetes, and other related disorders.

The problem of hyperplasia in severe obesity in man continues to be a paramount concern since hyperplasia is the morphological basis for the intractibility of the disorder. Fat cells, once formed, persist indefinitely. Faust (AM 20508) has demonstrated differential hyperplasia in hyperphagic rats (VMH lesioned) due to differences in diet composition even when gaining equivalent weight. Pair-gained rats fed a milk diet had a larger number of adiposites than did chow-diet fed controls. High fat feeding was shown to induce adipocyte hypertrophy in all fat depots as well as hyperplasis in some. Starved rats or rats exposed to cold had reduced fat cell size, but showed no reduction in number of fat cells. Diet restriction during early life resulted in less fat mass and fewer fat cells, characteristics which were sustained in later life when food intake was no longer restricted. Thus, the composition of the diet and levels of energy intake in early life appear to be important factors in human obesity.

Studies are being supported on the physiological controls of homeostasis. McHugh (AM 19302) has shown for both man and monkey that the emptying rate of glucose from the stomach is related to the amount of nutrient load delivered to the duodenum and duration of gastric inhibition produced by the nutrient delivered. Intravenous perfusions of small amounts of cholecystokinin (0.7-3 picamoles/kg/min) not only inhibited gastric emptying time in monkeys but reduced food intake intake as well. These observations support the hypothesis that the satiety effect of cholecystokinin is mediated through its inhibitory effect on gastric emptying. Such studies have the potential of defining fundamental mechanisms which may be disturbed in such pathological conditions as obesity, diabetes mellitis, atherosclerosis, and peptic ulcer.

Romsos, Michigan State University (R01 AM 15847), in studies designed to explore the role of Na+K+ATPase in thermogenesis and obesity, has found that ventral medial-hypothalmus (VMH) lesioned rats, which overeat and become obese, have reduced levels of Na+K+ATPase in skeletal muscle than do sham operated controls. This finding supports a previous observation that obese (ob/ob) mice had lower levels of muscle sodium than lean counterparts. It is postulated that Na+K+ATPase may contribute significantly to cellular thermogenesis.

In an other work by Glick (AM 27019) on the effect of diet composition on weight of brown adipose tissue (BAT) in rats, the level of dietary fat had no effect on BAT, whereas a low protein high carbohydrate diet was most effective in producing hypertropy of the BAT. Studies on the effect of meal pattern on efficiency of energy utilization by workers at Michigan State (AM 15847) revealed that meal pattern has a minimal effect on body fat control provided the control animals are pair-fed throughout the entire experiment. Their work also indicated that obese mice require more Vitamin E than do lean mice.

The neurotransmitter control of experimentally induced food and water intake in man has been studied (Campbell, AM 20494) by infusing appropriate neurotransmitter receptor agonists and antagonists. High doses of naloxone, an opiate receptor blocking agent, did not reduce experimentally induced hunger and thrist, but food intaked was decreased significantly. These results implicate opiate receptors in a satiety mechanism for feeding, such as by decreasing gastric emptying.

Studies on hunger and thrist response to 2-deoxy-D-glucose (2DG) suggest that the increased water intake caused by 2DG does not depend on elevated hunger and demonstrate that increased caloric intake after 2DG infusions is not dependent on greater thrist.

The beta-adrenorecepter blocker, propanolol, was administered to examine the sympathetic nervous system mediate glucose-induced thermogenesis. No effect was found in men, but propanolol appears to inhibit the increase in thermogenesis in women. Preliminary results also indicate that obese diabetic patients with severely impaired glucose tolerance appear to have subnormal increments in meal induced thermogenesis. The possible interrelationship of dietary induced thermogenesis and selective induction of sympathetic nervous system activity by carbohydrate, which may be sex dependent, is intriguing and provocative. Also, since this series of events was found to be insulin dependent, one might speculate that the propensity to develop and maintain the obese state, as commonly seen in diabetes, is related to absent or diminished dietary induced alterations in energy expenditure.

Controlled studies in 19-44 year old men revealed no change in blood lipids and lipoproteins due to exercise when body weight was kept constant. Exercise associated with weight reduction decreased VLDL cholesterol 17% and increased HDL cholesterol 12%, while weight reduction through caloric restriction increased HDL cholesterol by 24%, but VLDL cholesterol also increased.

Other basic studies in rats with insulin (Stern, AM 18899) suggest that, while hyperinsulinemia is not sufficient to explain why certain animals become obese, hyperinsulinemia can play a significant role in the development of obesity. One aim of drug therapy in obesity should be to lower hyperinsulinemia where it exists.

The role of the hypothalmus in weight regulation, energy balance and feeding behavior is receiving considerable research attention. Experimental manipulations which produce similar overall effects on caloric intake can lead to very different patterns of nutrient choice. Glycerol seems to play a special role because, mole for mole, it is more effective in producing anoxeria than either glucose or beta hydoxybutyrate (Davis, AM 26030). Relationships between food and brain amino acid concentrations and brain neurotransmitter concentrations appear to be important in determining eating behavior. Plasma and tissue amino acid concentrations are known to be altered by such disorders as diabetes and hepatic failure as well as by diet.

A pancreatic polypeptide appears to be deficient in genetically obese mice (Kimmel, AM 26191). Recent work suggest that obese humans also may be deficient or hyposecreters of this hormone.

Intestinal bypass for morbid obesity has undergone close scrutiny by the medical profession as an alternative to medical management or surgical gastric bypass in these difficult patients. Studies are underway on the mechanism responsible for hepatic injury in animals following this operation.

The Nutrition Program administered 56 active research grants and one core center on obesity research amounting to 4.8 million dollars, including 1.24 million supported from Diabetes Program Funds.

2. Minerals

Twenty three grants, amounting to 1.6 million dollars, were active in FY81. These related to the following minerals: iron (4), copper (3), zinc (5), selenium (5), chromium (1), silicon (1), molybdenum (1), magnesium (1), tin (1) and aluminum (1). Much progress has been made on the role of zinc, chromium, and selenium in metabolism of animals and man.

It is likely that trace mineral imbalances and deficiences may be involved in the cause of chronic, adaptive diseases. To this end, The Nutrition Program is supporting a workshop to develop guidelines for the systematic collection of trace mineral content of various human tissues.

Iron deficiency remains one of the most common forms of malnutrition and is particularly prevalent in rapidly growing infants and children. Studies by Dallman (AM 13897) with mature rats fed ironlow diets showed that hematocrit was decreased before liver storage iron fell to its lowest concentration. Cytochrome C levels in the intestinal mucosa were depressed even before significant anemia had developed. Depression of skeletal muscle cytochrome C and myoglobin was proportionately less profound in mature than in younger animals. More research is needed to evaluate the health significance of iron deficiency with emphasis on work performance and susceptibility to infection.

Studies on mineral intake in five free living male subjects for 20 days (King, AM 10202) yielded averages of 13.1 mg of zinc, 1.65 mg of copper, 17 mg of iron, 438 mg of magnesium, and 1.27 gms of calcium intake per day. Of these, intakes of zinc and copper were the least adequate being below two-thirds of the RDA on about 30% of the days. Since the ratio of zinc to protein generally has been found to be 1.4 gm zinc per 10 gms of protein consumed, it would require very high protein diets to reach the present RDA. The prudence of this needs to be evaluated.

Selenium is an essential micronutrient required for the function of the hydrogen-peroxide decomposing enzyme, glutathione peroxidase. However, little information is available as to how to evaluate the selenium status of people. Negative correlations have been reported between blood selenium levels in people and certain forms of cancer and cardiovascular problems. Recent identification of Kesham's disease in a peasant population in mainland China is the first demonstration of a possible deficiency of selenium in humans. The average intake of selenium in the U.S. is estimated to be 150 micrograms, about half the level that some advocate for optimal health. More information is required.

Abberations in metabolism of copper are at the root of two well-characterized afflictions of humans - Wilson's Disease and Menkes' Syndrome. Problems in lysyloxidase function, perhaps due to marginal copper insufficiency, may cause other less well-characterized disorders of connective tissue. One investigator (Harris, AM 26604) has focused on the role of ceruloplasmin in the regulation of lysyloxidase. Disorders of copper metabolism may be associated with a failure to transfer copper to ceruloplasmia, which in turn has been shown to deliver copper to lysyloxidase. Another scientist (Bonner, AM 26174) has demonstrated genetic differences in the copper binding protein content of duodenal tissue in mutant strains of mice. Studies by Greger, U. of Wisconsin (AM 25666) revealed that the amount of additional tin (50 mg) provided by four one-half cup servings daily of acidic fruits packed in unlacquered cans was sufficient to depress the absorption and retention of zinc by adult human subjects. This effect could be important among pregant women and young children who are consuming marginal levels of zinc and high amounts of canned fruit juices.

3. Vitamins

Research advances are being made in understanding the mechanism of action of vitamins in specific metabolic pathways.

Goodman (AM 05968) has obtained important new information on vitamin A transport and retinal-binding protein metabolism, the enzymatic hydrolyses of retinyl esters in the liver, intracellular binding proteins for retinol, and the transport of vitamin D and and 25-hydroxyvitamin D in human plasma. Humans with renal disease may become deficient in the active form of vitamin D (1,25 dihydroxy vitamin D_3). Studies by Wassner (AM 24061) indicate that both vitamin D deficiency and reduced kidney function may reduce protein synthesis and increase protein degradation in skeletal muscle in rats.

Based on studies of Norman and co-workers (AM 14750), it appears that appropriate application of the assays for three vitamin D metabolites (25 hydroxy vitamin D; 1,25 dihydroxy vitamin D, and 24,25 dihydroxy vitamin D) can yield insights in both the normal handling of vitamin D as well as the perturbations in disease state. Plasma clearance of the 1,25 dihydroxy form is unusually rapid (2-3 hours) while that of the 24,25 dihydroxy form is quite slow (14 days). These investigators have observed an impaired production or breakdown of 1,25 dihydroxy in patients maintained on total parenteral nutrition. Further studies are needed to learn the significance of this observation.

De Luca and co-workers (AM 14881) has isolated a new pathway of vitamin D metabolism. This group has isolated and identified a new metabolite - 25-hydroxy vitamin D_3 26,23 lactone. It appears that conversion of 25-OH-$D_3$to 23,25-$(OH)_2D_3$ and subsequently to 25-OHD_3 26,23- lactone is a major metabolic pathway in the kidney. The latter compound is biologically inactive in intestinal calcium transport, bone calcium mobilization and the mineralization of rachitic bone. Its role remains unknown.

Active forms of vitamin D, discovered, identified, and synthesized in De Luca's laboratory have been shown to be effective in treating metabolic bone disease including renal osteodystrophy, hypoparathyroidism and psuedohypoparathyroidism. Continued studies designed to understand how they function and how they are metabolized can be expected to yield important information which could optimize their use in treating bone disease.

De Luca's laboratory also is searching for the active forms of vitamin A in the hope of learning which compounds are responsible for epithelial differentiation or differentiation of bone tissue. Identification and chemical synthesis of these compounds could provide important new tools for treatment of skin and bone disorders.

Efforts have been continued by Suttie (AM 14881) to determine the properties of liver microsomal vitamin K dependent carboxylase and the vitamin K 2,3-epoxide reductase. Drug interactions with the vitamin K epoxide reductases also were studied as a means of better understanding the clinical use of coumarin anticoagulants to antagonize their synthesis.

The demonstration of the widespread occurrence of a pregnancy - specific riboflavin-binding protein in animals and the identification of a specific immunoglobulin G subfraction, which appears to serve this function in humans, is a good example of a significant gain in fundamental knowledge of real relevance to human health and disease.

Much has been learned about vitamin B_6 phosphate oxidase in terms of development, control, and the mechanisms by which it operates. The flavocoenzyme requirement of this enzyme and the fact that riboflavin status affects its activity does much to explain the subcellular, cellular and even macroscopically observed vitamin B_6 deficiency attendant to riboflavin deficiency in the mammal (McCormick, AM 25746). This oxidase potentially allows for a facile biochemical assessment of riboflavin in humans, since assays can be done on blood cells.

The lack of a suitable animal tor studies on vitamin C metabolism has limited our knowledge about the body's need for ascorbic acid in various physiological states and relation of ascorbate nutriture to the etiology and treatment of various diseases. Riviers (AM 25996) has developed methodology for kinetic analysis of ascorbate metabolism in the guinea pig and has characterized the two-pool model. Ingested ascorbic acid has been demonstrated to have profound effects on size of body pools, half-life and turnover rates of the vitamin. These accomplishments have greatly expanded our understanding of ascorbate distribution and metabolism and offer an experimental animal model for further studies.

Studies by Hall (AM 17767-08) show that cobalamin has a role in leukocyte function and presumably in resistance to infection. Cobalamin affected protein synthesis and cell division of lymhocytes, as well as the hexose monophosphate shunt activity of human granulocytes.

4. Proteins and Amino Acids

The Nutrition Program supports 29 active grants involving research on protein and amino acids, totaling 2.3 million dollars. These include studies on protein and energy utilization and requirements in man, nutritional regulation of protein metabolism, intestinal transport and metabolism of peptides, amino acid and protein nutrition in uremia, methodology for assessing protein nutritional status in man, dietary lysine and carnitime formation, and the effect of diet and purine metabolism in gout and malnutrition.

A very interesting development is the use of stable isotope probes (13$_{C}$ ^{15}N and ^{2}H) for quantifying whole body fluxes of indispensable amino acids and their major components. Preliminary work suggest that it should be possible to use these labeled amino acids to more accurately determine dietary protein and/or amino acid requirements of individuals (Young, AM 15856).

Children with protein-calorie malnutrition exhibit unusual susceptibility to infection. Several host-defense mechanisms are depressed, including phagocytosis and killing function of leukocytes, cell mediated immune response, inflammatory response and antibody production. Recent reports also suggest that protein-calorie malnutrition adversely affects a child's complement system.

Recent research reveals that pregnant primates can withstand diets containing as little as 3.35% protein (caseion) without obvious ill effects to themselves. Moreover, the offspring were normal when tested by numerous physiological, behavioral and growth measures. The investigator has concluded that most primates are well adapted to withstand low protein intakes during pregnancy. Babies also appeared to adapt if they are well-fed after birth. Additional studies are being made of these infants' skeletal growth.

Work in Peru has shown that wheat, potatoes or sorghum flour alone are not adequate in protein to support normal infant growth. Potatoes appeared to be first-limiting in the essential amino acid, methionine. When 50% of the calories and 80% of the protein in the diet was supplied by wheat and 20% of the protein from casein, excellent growth was supported although plasma amino acid levels indicated that lysine was still a first-limiting amino acid. However, when lysine was added, wheat could be used to supply all the protein and 75% of the calories in the diet with no adverse effects on growth.

The branched chain amino acids, leucine, isoleucine and valine make up about 40% of the daily requirement of indispensable amino acids in man. Recently it has been found that, in addition to being essential substrates for protein synthesis in tissues, branched chain amino acids serve as biochemical regulators and precursors in a number of complex reactions. These reactions include cholesterol synthesis, protein turnover in skeletal muscle, oxidative energy metabolism in muscle, gluconeogenesis, insulin secretion and urea formation. Since branched chain amino acids regulate protein turnover in muscle, these amino acids are being studied in treatment of conditions of protein wasting, such as trauma and sepsis. Since branch chain amino acid also regulate amino acid uptake by the brain, these amino acids are used also in treating hepatic encephalopathy and phenylketonuria.

Mitch (AM 26903) has found nutritional therapy consisting of a low-protein diet plus supplements of essential amino acids or their nitrogen-free analogue to be effective in treatment of patients with chronic renal failure. Serum proteins and serum chemistry values are maintained within the normal range. In some cases, nutritional therapy appears to delay the rate of loss of renal function, presumably because the alpha keto analogue of leucine (alpha ketoisocaproate) has been shown to decrease nitrogen wasting of starving individuals.

5. Dietary Fiber

The Nutrition Program supports four active research grants directed at the study of dietary fiber. These relate primarily to determining the chemical characteristics of various fiber components, development of appropriate methodology for measuring detary fiber and evaluation of the forms and sources of dietary fiber components on bioavailability of nutrients.

Research results from U. of Wisconsin (Marlett, AM 21712) suggest that some foods will require short periods of amylase treatment in measuring neutral detergent residues, as the values obtained with the unmodified Van Soest ND procedure are significantly lowered. Also, fecal neutral detergent fiber (NDF) appeared to contain other unidentified components. To date, it appears that the Saeman hydrolysis procedure, as modified by Selvendren, may be a suitable means of hydrolyzing food and fecal NDF. At present there are many discrepancies among studies on the effect of dietary fiber on mineral bioavailability. Some natural fiber sources are reported to have marked adverse effects on bioavailability of minerals, and others very little. These differences may be due to variations in fiber composition of natural fiber sources. Studies are underway to compare the effects of cellulose, xylan, pectin and cornbran on the bioavailability of calcium, magnesium, phosphorus, iron, copper, zinc and selenium. Stable isotopes of iron, copper and zinc will be fed during the feeding of cellulose and phytic acid to assess better the bioavailability of these minerals (King, AM 10202).

6. Lipids

Nine research grants on lipids were active in FY81. Of these, three dealt with essential fatty acids and six with absorption and metabolism of lipids.

Meng (AM 18281) is investigating the effect of an essential fatty acid (EFA) deficiency on prostaglandin synthesis or production of arachidonic acid metabolites in blood platelets, as these may be earlier signals of EFA deficiency. With the biochemical criterion (i.e. tetraene/triene ratio of plasma lipids) for the diagnosis of EFA deficiency lowered from 0.4 to 0.2, the incidence of EFA deficient patients requiring TPN remains high (33%). Better information is needed concerning dosages of fat emulsion or linoleic acid for prevention or correction of this condition during TPN.

Connor (AM 29930) is determining the essentiality of dietary omega 3 fatty acids in primates. The existence of a dietary requirement for omega 3 fatty acids remains an unresolved issue in human nutrition, though recent work has shown that omega 3 as well as omega 6 fatty acids are precursors for the syntheses of prostaglandins and related substances. These substances have wide-ranging physiological effects, mediated through effects on adenyl cyclase activity or on ion transport in membranes. Human infants fed cow's milk and infant formulas have far less 22:6W3 in blood lipids than do infants receiving human milk. In the cerebral gray matter of both humans and rats, 22:6W3 accounts for one-third of the fatty acid content of ethanolamine and serine phosphoglycerides. Information on primates should provide a better basis for assessing the importance of omega 3 fatty acids in human nutrition.

National Digestive Disease Education and Information Clearinghouse

The Digestive Diseases Clearinghouse is an information service of the National Institute of Arthritis, Diabetes, and Digestive, and Kidney Diseases of the National Institutes of Health. The Clearinghouse acts as the coordinator of a national effort to educate the public, patients, their families, physicians, and other health care providers about digestive health and diseases, information is provided on symptoms, diagnosis, treatment, and prevention as well as research.

The Clearinghouse provides a central point for the provision and exchange of information. In cooperation with the professional organizations, foundations, and voluntary health organizations involved with digestive health and diseases, the responsibility of the Clearinghouse is three-fold. It will aid the distribution of available educational and informational material. It will identify needed materials. And, it will encourage production of these needed materials.

In response to the Digestive Disease Commission recommendations, submitted to Congress in 1979, the Institute began the developement of the Clearinghouse early in 1980. The professional and lay organizations concerned with digestive diseases were asked to name individuals to advise the Clearinghouse. A contract was negotiated with InterAmerica Research Associates to begin the operation of the Clearinghouse. In consultation with the digestive diseases professional and lay organizations, plans were made for the focus of the Clearinghouse for the first year of operation.

In December 1980, the Health Programs Extension Act of 1980 (S988, Section 440) mandated the Clearinghouse as follows.

> "The Secretary, acting through the Director of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, shall establish an information and education center for digestive diseases (1) to identify, collect, analyze, and disseminate information respecting the diseases, and (2) to serve as a national educational resource for patients with such diseases and their families, physicians, and other health professionals."

Two meetings have been held with the advisors to the Clearinghouse in which plans for the Clearinghouse have been developed, and attainable goals for the first year of operation have been identified.

Three fact sheets on the following topics were produced, "Cirrhosis of the Liver" written by Willis Maddrey, M.D., Johns Hopkins Medical Center and Hymen Zimmerman, M.D., George Washington Medical Center, "Diarrhea, Infectious and Other Causes" written by Sidney Phillips, M.D., Mayo Clinic, and "Heartburn" written by Donald Castell, M.D., Uniformed Services University in the Health Sciences. Other topics have been identified and authors selected and contacted to write the fact sheets. These topics include "Milk Intolerance" to be written by Gary Gray, M.D. One "Letter" from the Clearinghouse was produced, a "Directory of Organizations", and a flyer describing the Clearinghouse activities were produced.

KIDNEY UROLOGIC AND BLOOD DISEASES PROGRAM
NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

Nancy Boucot Cummings, M.D.
Associate Director, NIADDK

The Kidney, Urologic, and Blood Diseases Program (KUBD) of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) continues to focus primarily upon research into the basic mechanisms of biologic processes and diseases of the relevant disciplines and subcategories of nephrology, urology, and hematology. The emphases within these areas are upon developing an understanding of fundamental processes in the physiologic and pathophysiologic states so that rational treatment may be devised. The ultimate goal of such research is to find means to prevent or to arrest the underlying etiologic causes of the diseases falling within the program's responsibilities. The major support mechanism is the investigator-initiated research grant. There is a modest investment in other related mechanisms: Program projects, research career development and training. The effort to diminish the use of contracts for research continues and this mechanism now is utilized primarily for developmental types of projects. Coordination in areas of overlapping and complementary interests continues to be maintained and nurtured with other institutes of NIH; with other governmental agencies; outside groups, both lay and professional; and with scientists in the respective fields. Assessment and evaluation of research and of program activity and productivity are maintained by program staff in consultation with outside experts when indicated, and within the limits placed by modest resources.

The two broad efforts to assess the research activity in the disciplines and directions in which research might be encouraged to expand or to curtail its efforts, provide an ongoing source of guidance and recommendations. Research Needs in Nephrology and Urology, the NIADDK sponsored study completed in 1978, is a resource which continues to indicate directions for encouragement of scientific research to be nutured. Mechanisms used include: Program Announcements of which three were issued this year--a broad based one addressing research interests in Kidney-Urology and one in Continous Ambulatory Peritoneal Dialysis; a broad based Program Announcement relating to diabetes melletus, the Kidney and Kidney Disease, issued jointly with the Diabetes Program; meetings and workshops of which two were sponsored by the Kidney and Urology Program; requests for contract proposals; and formal and informal discussion at scientific meetings. The Evaluation of Research in Hematology, initiated by NIADDK and supported by a contract awarded to Dr. Ernest Jaffe, Albert Einstein School of Medicine, has proven useful even while in progress to those institutes with primary research support in hematology. This hematology study will be published within the next year.

The combined emphasis upon investigator-initiated research and concern for finding means to prevent disease are indicated in the two KUBD sponsored meetings: Chronic Renal Failure Conference, March 1981, which focussed upon research into two of the main causes of chronic renal failure: cystic kidney disease and glomerulonephritis; and upon special problems of the young child with renal failure; and a conference on Renal Effects of Drugs and Environmental Toxicants, March 1981, which highlighted many potentially preventable causes of renal failure. Three clinical trials are underway as investigator-initiated research grants focussed upon: 1) glomerulonephritis treatment, 2) plasmapheresis as an intervention technique for lupus nephritis, and 3) surgical versus medical therapy of reflux nephropathy in children. Means to monitor clinical trials supported as investigator-initiated grants rather than as contracts have been developed.

The Specialized Centers of Research (SCORs) into Urolithiasis have been converted to Program Projects since their composition technically fell within this research mechanism. Their research productivity has continued to be high and responsible underlying mechanisms for stone formation can be assigned for about 80 percent of kidney stone patients. An effort to assess the validity of the SCOR mechanism to encourage research has begun with an RFP for evaluation of SCORs as a means to stimulate research which includes a parallel assessment of whether the serendipitous support of an area such as urolithiasis is riding a tide of increasing interest in an area or actually does increase research productivity by the extra encouragement, as compared to other possible mechanisms.

In an effort to encourage the highest quality of research applications and to emphasize areas of research needs, at a number of scientific meetings panels or talks have been given about the breadth of kidney research support, the areas of research promise, the relevance of research to costs of diseases such as end stage renal disease, and the research grant application process. Among such were those to: National Kidney Foundation/American Society of Nephrology; Society of University Urologists; American Urologic Association; American Society of Artificial Internal Organs. Analysis of long-term support of research into topics related to Chronic Renal Failure indicated fairly level support over the a ten-year period and portrays research potential in chronic renal failure, at both fundamental and clinically applied levels of studies.

The Hematology Program continues its cooperative efforts with the National Heart, Lung and Blood Institute informally and through the Coordinating Committee for Blood Related Activities. The Iron Chelator Development Program which received its initial stimulus from interest in developing compounds for treatment of Cooley's Anemia patients has provided at least three compounds which are promising enough to require animal toxicologic testing, the topic of a request for contract proposals that should result in the award of a contract in FY 1982. The reports of hemosiderosis in chronic renal failure indicates new clinical applications for iron chelators in a larger patient population than that of Cooley's Anemia.

Coordination and cooperation between institutes is exemplified by the joint sponsorship of the conference on Renal Effects of Drugs and Environmental Toxicants with the National Institute of Environmental Health Sciences and of the planned meeting on Neurosciences and Uremia with the National Institute of Neurological and Communicative Disorders and Stroke, as well as cooperation on a day-to-day functional level. The most active interagency cooperation has involved that necessary with the many levels of committees involved the end stage renal disease which include The Heath Care Financing Administration, The National Center for Health Care Technology, The National Center for Health Statistics, etc. Concern with problems relevant to affirmative actions has extended from training courses in this area to presentations about relevant topics both within NIH and to outside groups, as well as encouragement of the goals of the Equal Employment Opportunity programs.

HEMATOLOGY PROGRAM

The Hematology Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases supports investigation into basic mechanisms of normal blood cell function and into pathogenesis of hematologic disorders, and includes development of treatment modalities, clinical application and evaluation of treatment. This research increases both fundamental and applied knowledge about blood. The availability of blood and blood cells facilitates study of membrane phenomena, protein structure and function, etc. to obtain information broadly applicable to medical problems such as chronic renal insufficiency, transplantation, arthritis, nutritional disorders, and malignancy.

Major hematologic categories supported are:

1. anemias of genetic origin, particularly the hemoglobinopathies, with emphasis on regulation of hemoglobin and hemoglobin synthesis (e.g., Cooley's anemia, sickle cell anemia).

2. metabolic disorders, related to iron transport and storage (hemosiderosis), porphyrin metabolism (porphyrias), and enzymatic defects (e.g., red cell glucose-6-phosphate dehydrogenase deficiency).

3. nutritional anemias, especially those involving iron, folic acid, and vitamin B_{12}.

4. disorders of blood cell production, such as those encountered in systemic and/or metabolic disorders. Examples are the anemia of chronic renal failure, aplastic anemia, and polycythemia.

5. immunology, of hematopoietic tissue transplantation and of autoimmune hematologic disease.

A major difficulty was experienced in Fiscal Year 1981 with the funding of research grants in Hematology, due to the commitment of a relatively large percentage of appropriated funds for the continuation of grants funded in previous years, combined with the receipt of the largest number of approved research grant applications in the program's history. In addition, numerous administrative uncertainties throughout the year regarding the budget led to problems with planning and in advising applicants.

Anemias of Genetic Origin

Approximately 40 active research grants support investigations involving largely the sequencing of animal globin DNAs and the analysis of mechanisms controlling the switch in humans from the synthesis of fetal hemoglobin to adult forms as a possible cure for thalassemia. Application of recombinant DNA technology to problems of the regulation of hemoglobin biosynthesis continues to stimulate investigation. Using these techniques, the characteristics of globin gene transcription in a number of patients with β^+-thalassemia have been analysed, with results demonstrating that the defect in β^+-thalassemia resides not at the transcriptional level but at the post-transcriptional, or mRNA processing, level of gene expression. Indications are that the defect in some patients involves abnormal processing and accumulation of nucleotide sequences complementary to the large intervening sequence of the β gene. In related studies, the structural analysis of the β^+ thalassemic globin gene has been completed, with the result showing that the only difference detectable from the normal β globin chain is a single nucleotide change. This change probably results in an unstable mRNA which is degraded rapidly, thus reducing the amount of functional mRNA.

Studies of globin gene sequence studies of the three fetal γ globin genes, have revealed an unexpected mechanism by which duplicated genes co-evolve in an organism. The data indicated that the fetal γ globin genes have exchanged DNA along the chromosome, by intergenic exchange between G γ and A γ gene loci. Through cooperative efforts of several laboratories, the complete nucleotide sequences of the embryonic ε, and adult β and δ genes have been determined, and comparisons are being made of the DNA sequences of all the β -like globin genes of man. This new understanding about genes may provide an anatomical basis for planning experiments about the nature of mechanisms controlling the activity of human embryonic, fetal, and adult globin genes.

Metabolic Disorders

Several supported investigators are studying to define the role of genetic and environmental factors in the pathogenesis of certain of the porphyrias. Porphyria cutanea tarda (PCT), the disorder of porphyrin metabolism most often seen, is characterized by the hepatic synthesis and urinary excretion of excessive amounts of porphyrins. Clinically, PCT, is identifiable by a photosensitive dermatitis, as well as by abnormalities of iron metabolism leading to hemochromatosis. The removal of iron by phlebotomy or by the administration of iron chelating agents induces both clinical and biochemical remission in PCT patients. Recently, using in vitro systems, it has now been determined that iron inhibits the activity of two enzymes in the metabolic pathway of hepatic porphyrin synthesis. Further, it has been found that the activity of one of the two enzymes, uroporphyrinogen decarboxylase is significantly lower in tissues from PCT patients from several different families than it is in normal humans. These findings have been confirmed, and it is hoped that the information will lead to a better understanding of the mechanism of PCT with improved prospects for therapy.

In another area of red cell metabolic disease, that of phosphofructokinase deficiency, it has been shown in one patient that this disorder results from the homozygous deficiency of one of the five tetrameric isoenzymes of the red cell. Additional patients with this disorder are being investigated to define new variants and enzyme patterns.

Nutritional Anemias

Several NIADDK supported investigators are studying problems associated with the absorption, transport, storage, and interactions of various trace metals including copper, cobalt and iron, as well as vitamin B_{12} and folic acid.

Using animal model systems to investigate the interrelationships of the gastrointestinal pathways for iron, lead, and cobalt, competition among these elements is evident. Each is absorbed primarily in the duodenum, and each inhibits the absorption of the others when present. For example, the absorption of cobalt and lead are increased in iron-deficient animals and diminished in iron-loaded animals. Certain of the methods used to affect iron absorption, such as acute bleeding and dietary iron deprivation, result in increased iron absorption but do not appear to affect the absorption of lead and cobalt. This finding suggests that alternative pathways for iron absorption exist.

Nitrous oxide (N_2O) is one of the most widely used anesthetics and generally is believed to be inert and safe. Recent research has shown that N_2O is associated with hematologic and neurologic disorders that mimic cobalamin deficiency. These studies raise the concern that high concentrations of N_2O may have a role in the increased incidence of spontaneous abortions, birth defects, and possible malignancies observed in anesthesiologists, dentists, and in operating room and dental personnel. In the liver, most of the cobalamin is bound to two enzymes, methionine synthetase (MS) and methylmalonyl-CoA mutase (MMCoAM). Using specific enzyme assays, it has now been found that as little as 2% N_2O results in 20% inhibition of MS activity, with a lesser inhibition of MMCoAM. Recovery of MS activity was slow and incomplete at 6 days. Higher doses of N_2O result in nearly complete inhibition of these enzymes. Cobalamin analogues appear to be formed under such conditions, and are preferentially excreted, leading to cobalamin deficiency and decreased enzyme activity. Experiments are underway to confirm this process and to define its clinical implications.

Disorders of Blood Cell Production

Disorders of blood cell production continues to be one of the most active areas of investigation in hematology. A large number of scientists are investigating the basic mechanisms involved in the regulation of erythropoiesis, and trying to apply the information obtained to human hemopoietic diseases. A serious shortage of purified human erythropoietin still exists. There has been a limited increase in the amount of information available about the chemical structure of erythropoietin.

The regulatory mechanisms of cell proliferation and differentiation by mammalian hemopoietic tissues are poorly defined. Many of the studies underway address the issues of humoral and cellular controlling factors of proliferation of erythroid progenitors in vitro. Hemopoietic progenitor cells are obtained easily and techniques are available for cloning these cells in vitro, making it possible to obtain data widely applicable to understanding of disorders involving other less available tissues, as well as bearing on such clinical problems as the anemia of chronic renal failure.

The mechanisms controlling the switch in erythropoietin production from liver to kidney and which accompany growth and development of the fetal and newborn mammal have sparked investigations. Using anemic fetal and newborn sheep which were either hepatectomized or bilaterally nephrectomized, research workers have compared erythropoietin activity of blood samples to the levels present in plasma of either anemic intact fetuses or of anemic intact newborns. The timing of the switch appears variable, but generally it occurs over a 10-day period beginning at about day 20 post-partum. The switch is not affected by hormonal agents which influence erythropoietin production in the fetus but is profoundly delayed by reduced tissue oxygen availability during the fetal-newborn period. A better understanding of the factors influencing this switch may contribute to the design of rational therapeutic approaches to the anemia of renal failure.

Immunohematology

This research area of the Hematology Program has the goal of developing information on the genetic and cellular mechanisms underlying natural resistance to foreign and parental hemopoietic cells, normal and neoplastic. It has potential for understanding cellular interactions, the immunobiology and immunogenetics of hemopoietic cell transplantation, and the elucidation of the pathogenesis of blood disorders. Evidence which strongly suggests that natural killer cells are the source of hybrid resistance to parental bone marrow grafts *in vivo* has been obtained from studies on inbred strains of mice. Such reactivity of natural and induced crystotoxin lymphocytes against self has great significance for achieving an understanding of the regulation of cellular interactions.

Iron Chelator Development Program

A request for Contract Proposals has been issued for toxicologic evaluation in animals of new iron chelating compounds previously screened in the hypertransfused rodent. At least three compounds initially appear promising enough to undergo such studies, and it is hoped that a toxicology contact may be initiated early in FY 1982.

KIDNEY DISEASE AND UROLOGY PROGRAM

The funds administered by the Kidney Disease and Urology Program during FY 1981, including the support for investigator-initiated research grants, ROls (291), program projects (12), contracts (16), Clinical Investigator Awards (18), and Research Career Development Awards (18) exceeded $37,763,000 while the support for National Research Service Awards, both individual (21) and institutional (29) amounted to $1,950,000.

Manpower Development

Four of five competitive renewal institutional National Research Service Awards (T32s) were funded in FY 1981, for a total of 29 active training programs supported by the Kidney Disease and Urology Program, which provide stipends for 92 postdoctoral and 4 predoctoral trainees. Added to the 13 active individual National Research Service Awards (F32s) were 8 new, for a total of 21 fellowships supported with FY 1981 funds. Five new Research Career Development Awards were established, bringing the total to 18 and the Clinical Investigator Award Program has escalated to 18, with the addition of 6 new commitments.

Conferences

Awards were provided for partial support of the following conferences/workshops/symposia by the Kidney Disease and Urology (KDU) Program during FY 1981: (1) Arachidonic Acid Metabolites and the Kidney, June 4-5, 1981, Rome, Italy (2) International Symposium on the Brattleboro Rat, September 5-7, 1981, Hanover, New Hampshire, and (3) Advances in Systemic Lupus Erythematosis, October 2-3, 1981, New York, New York. A Chronic Renal Disease conference sponsored by the KDU Program was held March 5-6, 1981 and included half-day sessions on (1) Cystic Diseases of the Kidney, (2) Control of Treatment Morbidity and Uremic Toxicity with Hemodialysis and Hemofiltration Therapy, (3) Chronic Renal Failure: Unique Problems of the Child with Renal Failure and (4) Current and Future Trends in Research in Glomerulonephritis. A conference on Drugs and Environmental Toxicants, jointly-sponsored by the KDU Program of the NIADDK and NIEHS was held March 18-20, 1981 at Pinehurst, North Carolina. The program included topics such as acute renal failure models, nephrotoxicity of antibiotics, environmental toxins and interstitial nephritis, environmental toxicities and hazards and related immunopathological mechanisms.

RENAL PHYSIOLOGY/PATHOPHYSIOLOGY PROGRAM

The Renal Physiology/Pathophysiology Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases supports research on the structure and function of the kidney, such as metabolism, transport and fluid-electrolyte dynamics; diseases and mechanisms leading to chronic renal disease, including diabetic nephropathy, polycystic disease, etc.; specific immune mechanisms of kidney disease, including glomerulonephritis, interstitial and lupus nephritis; and effects of drugs, nephrotoxins and environmental toxins on the kidney. The following are highlights of some of the research activities supported by the Renal Physiology/Pathophysiology Program.

Glomerular Mesangium

The mesangium occupies an intercapillary position in the glomerulus and is a common site of immune complex deposition in human and experimental renal disease. Chronic mesangial injury has been associated with slowly progressive renal insufficiency. A group associated with the Regional Kidney Disease Program in Minnesota is studying the determinants of glomerular ultrafiltration in models of glomerular disease in which the principal site of injury is the glomerular mesangium. They have developed a reproducible model of chronic mesangial immune complex disease in the rat by administering intraperitoneally, 8 mg/100 gm body weight of ferritin, daily for 6 weeks. Their data demonstrate that chronic mesangial immune complex disease results in approximately a 20 per cent reduction in total kidney glomerular filtration rate (GFR) and in single nephron glomerular filtration rate (SNGFR). Assessment of the glomerular dynamic factors responsible for the reduction in SNGFR demonstrated that the glomerular capillary pressure was reduced 1.6 percent as a consequence of increased afferent and efferent anteriolar resistances.

In another model of experimental glomerular disease these investigators are evaluating the effects of alloxan-induced diabetes in the rat. These studies are being performed in two variations of the experimental model: Group 1 - alloxan induced diabetic rats that are allowed to remain hyperglycemic for 1 or 3 months and are studied at these time intervals. Group 2 - alloxan induced diabetic rats that are given small supplemental daily doses of insulin are studied after 3 months of the disease. This latter group was felt to be important since the untreated diabetic rats grow at a significantly slower rate than age-matched controls. It was observed that small supplemental daily doses of insulin allow for normal body growth despite hyperglycemia. Results of these studies demonstrated that in Group 1 diabetic rats there was a reduction in the GFR and SNGFR as a result of a decrease in the glomerular capillary pressure secondary to an increase in arteriolar resistances. Similar reductions in renal plasma flow (RPF) and glomerular plasma flow (GPF) were observed also. These changes occur in the absence of any detectable decrease in the intravascular volume and are present at one month of the disease, wherever functional changes occur before detectable changes in glomeruli are observed by light, immunoflourescence and electron microscopy. After three months of experimental diabetes, decreases in GFR and SNGFR are still evident and in fact are slightly more pronounced. These researchers attribute these changes to decreases in glomerular capillary pressure secondary to an increase in arteriolar resistance.

Lupus Nephritis

Renal failure due to severe lupus nephritis is an important complication of systemic lupus erythematosus. Patients with severe lupus nephritis at present are, usually treated with a drug regimen which itself carries a significant mortality. It is widely accepted that high dosages of steroids (such as prednisone), have improved survival, but it is still not established that further addition of a cytotoxic agent (such as cyclophosphamide) incrementally improves survival and preserves renal function. Plasmapheresis is widely used to treat various "immunologically mediated" diseases, but evidence supporting its efficacy is anecdotal or involves only retrospective evaluation. During the past few years several reports have appeared describing possible beneficial effects of plamapheresis in the management of lupus nephritis patients and suggesting that plasmapheresis may offer improved results over that of existing therapy. To gain a better understanding of whether or not plasmapheresis is a useful therapeutic modality, a controlled clinical trial, incorporating at least ten centers recently was funded to gain additional information about: 1) the effects and feasibility of plasmapheresis in general; 2) relationships between various histological abnormalities and the response to therapy; and 3) mechanisms of action of plamapheresis in lupus nephritis. Plans for the study include the entry of 200 patients over three years and for up to a five year patient follow-up. The patients will be allocated randomly either to a group receiving prednisone and cyclophosphamide or to a group receiving these drugs plus plasmapheresis. The study has been carefully organized and is being carried out by an experienced group of nephrologists and rheumatologists, who have worked with lupus nephritis and with plasmapheresis.

Serum Activating Factor in Glomerulonephritis

A serum complement activating factor in glomerulonephritis which is capable of activating C3 through the alternative complement pathway has been derived from a patient with membranoproliferative glomerulonephritis by a group at Rush-Presbyterian-St. Lukes Medical Center. Although the activation of C3 by this material was comparable to zymosan, it was significantly less than C3 nephritic factor. This new C3 activating factor hitherto undescribed, discovered by Dr. Edmund Lewis and associates, may play an important role in the pathogenesis of some forms of nephritis. According to leaders in the field, this finding adds a new dimension to the biological importance of complement activating factors in renal disease. Another exciting aspect of these studies is Dr. Lewis′ finding that beta-1-H globulin, an important regulatory protein, binds to the newly discovered activating factor. This could explain, in part, observations of low beta-1-H globulin levels seen in sera of patients with various forms of nephritic diseases by many investigators.

Active Sugar Transport in Renal Cells

A grantee of the Kidney Disease and Urology Program of the NIADDK at the University of Pennsylvania has reported a number of interesting findings that have evolved from his multi-faceted research program designed to study sugar transport and metabolism in renal tubules *in vitro* and in renal cells grown *in culture*. Some of his especially interesting findings are that: 1) insulin increases both glucose oxidation and gluconeogenesis; and 2) renal cells grown in tissue culture actively accumulate sugar. During the next five year funding period his studies are geared; 1) to elucidate the mechanism of insulin control of sugar transport and metabolism in renal cells by measuring the activity of key enzymes of carbohydrate metabolism in normal, hypoinsulinemia, and hyperinsulinemia suspensions of rat renal cortical tubules; 2) to study the effect of insulin on hexose transport and on gluconeogenesis in a renal epithelial cell line grown *in vitro*; 3) to study the mechanisms of renal fructose transport and metabolism in several newborn animals, such as piglets and lambs, as well as the effects of insulin on fructose transport and metabolism; and 4) to clarify the mechanisms of 2-deoxy-galactose secretions by the kidney and intestine using sugar analogs and measuring the actual driving forces of this process.

Renal Substrate Utilization in Relation to Function

Studies of glucose and lactate metabolism in the kidney underway at the University of Rochester are aimed at gaining an understanding of how substrates are utilized in the support of renal function. In certain disease states such as diabetes, nephritis or nephrosis, renal functional changes are observed. Therefore it is important to determine whether the changes in function are due to a defect in utilization of a substrate or to a primary defect in the function *per se*. Also, changes in the metabolic pattern, (that is, fates of a substrate) by which a substrate is utilized may occur in a disease state so that a further decrement in function will occur. For example in diabetes, glucose levels in plasma are high and may be attributed to an increased production of sorbitol in the kidney which can affect cellular function. By understanding the pathways by which a substrate is utilized in the kidney and how its metabolism is coupled to function, a more complete comprehension of how a disease affects overall renal function should evolve. These studies also may provide a more complete understanding of the energy requirements for the several work functions the kidney performs. In a recent study by this group, it was reported that there is a high rate of biosynthetic work which increases, in parallel with increases in sodium reabsorption. Thus, the energy requirements for sodium reabsorption are overestimated by not taking into consideration the rise in biosynthetic work which occurs when tubular function increases. These approaches employed by this group should also permit them to determine whether a hormone or neural mediator has a direct or indirect effect on kidney function or metabolism. Ultimately an understanding of how metabolism is coupled to and regulates renal function in health and in disease may be achieved.

Neural Control of Renal Excretion in the Primate

There is a growing interest in the role of the nervous system in the control of renal function, and whether its effects are exerted directly or indirectly through changes in extra-cellular fluid volume or hormone release. Currently available evidence suggests that the changes in diuresis and natriuresis may be mediated either by a direct effect on the renal tubule or by the release and inhibition of vaso-active substances, such as angiotensin II, prostaglandins or vasopressin. It is also known that activation of left atrial and of carotid baroreceptors results in both diuresis and natriuresis, but the former can be abolished by the exogenous administration of vasopressin. Furthermore, it is known that some of the neural effects are exerted upon the kidney through changes in intrarenal hemodynamics. In studies recently funded at the Texas A & M Research Foundation, an investigator will attempt to gain a more concise understanding of how the cardiopulmonary receptors and baroreceptors in the nervous system of primates control salt and water hemeostasis. Although these neural mechanisms have been implicated in the dog, there is very limited information in this area concerning primates. The three approaches involved in these investigations are: 1) to assess the role of the dorsal, spinal and baroreceptor nerves by various denervation procedures; 2) to assess the role of the vagal-aortic and carotid sinus nerves in the renal response to head-out water immersion versus non-immersion; and 3) to test the effect of cardiac denervation of the renal response to both head-out water immersion and non-immersion.

Phosphate Homeostasis in the Neonate

The concentration of inorganic phoshorous (Pi) in the plasma of the neonate, infant and young child is known to be substantially higher than that found in older children and adults and appears to be directly correlated with the rate of growth. However, the mechanism by which the high plasma levels are maintained in the developing child are not well understood. Based on the likelihood that the kidney plays a role in this phenomenon and that there is evidence to suggest that the renal response to various factors that regulate Pi handling vary with the stage of maturation, a pediatric nephrologist at the Albert Einstein College of Medicine is investigating this problem. To examine more closely the mechanisms of renal phosphate handling by the newborn kidney, phosphate titration studies are being performed in both the newborn and mature guinea pig. In reports of studies in which perfusate Pi concentration was increased gradually by adding neutral phoshate, while keeping the perfusate pH at 7.4, it appears that the reabsorption of Pi is directly proportional to the amount filtered up to filtered loads of 60-70 micrograms/minute. Beyond this range there is a tendency to plateau, suggesting that maximal sites of Pi reabsorption have been reached. When comparing rates of Pi reabsorption in the newborn and in the mature guinea pig over the same range of filtered loads, this investigator reports that the newborn guinea pig is able to reabsorb almost four times more Pi than the adult. The growing subject has an obvious need to remain in positive balance for phosphate and these data are supportive of the idea that the kidney is a key organ contributing to this phenomenon.

Toxic Effects on Renal Function

Experiments by a renal pharmacologist at Michigan State University were conducted to determine the effect of anesthesia on renal function in newborn piglets. Parameters that were measured included cardiac output, intrarenal blood flow distribution and plasma renin concentration as well as standard renal function tests. Results reported from these studies indicated that changes in renal and cardiovascular function due to anesthesia in the newborn are small. For example, no major differences were seen using a combination of nitrous oxide-oxygen plus ketamine or halothane or pentobarbital. These investigators suggest that whatever small differences that occur may be related to factors such as the degree of development of the autonomic nervous system.

X-RAY MICROANALYSIS

Although the use of x-ray microanalysis in renal research is subject to all the problems inherent in the use of a new technology, its promise appears to be great because its use for the localization of insoluble deposits in kidney tissue, the quantitation of micropuncture samples from tubular lumena and the localization and quantitation of histochemical reaction products are becoming standard procedures in a variety of laboratories involved in renal research. With its use, the validity of these procedures has been established and their further exploitation should lead to new research insights. Furthermore, the possibility of applying these technical achievements to renal research raises hope that a more precise understanding of the ion concentrations in lumena, interstitium, cells, and their organelles from various regions of the kidney in documented physiological and pathological states. Thus, elements to be identified may be naturally occurring, accumulated by some pathological process, or added to tissue as tracers or stains. Perhaps even more enticing is the anticipated concordance of the morphological and the biochemical in the identification and quantitation of the elemental content of soluble substances in renal cells, lumena, interstitium or even within cellular organelles using frozen tissue sections.

A group at the University of Texas at Houston that is exploiting the use of x-ray microanalysis is making attempts to gain an understanding of mechanisms causing renal damage in acute renal failure and in drug-induced nephrotoxicity and, in defining ways to ameliorate the toxic effects. For example, preliminary results reported indicate that clonidine (an antihypertensive agent) administration ameliorates mercuric chloride toxicity and that preventing potassium depletion decreases gentamicin nephrotoxicity.

UROLOGY PROGRAM

The Urology Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, supports research efforts into the basic mechanisms, pathophysiology and the therapeutic approaches to urologic disorders. These include: benign prostatic hyperplasia (BPH), urinary tract infection (UTI), urolithiasis, obstructive uropathy, neuromuscular dysfunction of the urinary tract, vesicoureteral and renal reflux, and sundry conditions such as Peyronie's disease, priapism, impotence, enuresis, congenital anomalies of the lower urinary tract, etc. Progress in research in a number of these areas is reported below.

Benign Prostatic Hyperplasia

Development of benign prostatic hyperplasia (BPH) is a common occurrence in men, and usually is present microscopically long before it becomes clinically evident. It is a major cause of morbidity in men over the age of 40, although uncomplicated it is not a leading cause of death. Recently there has been an increase in knowledge about factors influencing the development of BPH, primarily from studies in dogs. Very little has been added to knowledge about BPH in man.

Two grantees of the NIADDK working in collaboration, one at Northwestern University Medical School, and the other in Bicetre, France, continue their efforts to identify the nature, cellular distribution and concentration of steroid cell membrane receptors in epithelial and in stromal cellular elements present in BPH. This work is being correlated with the histological examination of unfractionated BPH tissue samples.

Efforts continue at the University of Colorado, in Boulder and in Denver, to correlate morphologic changes during the development of the prostate and the biochemical characteristics of this gland, with hormone cell membrane receptors. The focus of these experiments is primarily on recombinant techniques and upon cell membrane steroid hormone receptors measured by antibodies and by autoradiography. These investigators developed a germ-free breeding colony of athymic nude mice, which are host animals for BPH studies. Comparisons of the morphogenetic prostate pattern of fetal and of elderly male sources are made by growing specimens of human fetal prostate in these nude mice. Preliminary results indicate that nuclear androgen receptor binding is distributed over mesenchymal (connective tissue) cell nuclei. In contrast, epithelial androgen receptors are absent at this particular stage. The interaction between androgenic steroids and mesenchymal components of the prostate may account for the morphogenetic and biochemical differentiations of this gland. Ongoing recombinant experiments indicate that the adult prostate positively induces the formation of prostatic like structures upon embryonic epithelium derived from urogenital sinus or bladder.

At Johns Hopkins University, 44 beagle dogs of known age are being studied to correlate the exact time of the onset and development of BPH, and of changes in the physiological function of both testes and prostate, with age. A cross-sectional study of the effects of age on the male reproductive tract is being conducted under this protocol. The effects of different doses of testosterone on sperm production and upon prostatic function is being tested in these dogs. Androgen receptors are measured in cytosol and in nuclei of prostatic tissue from normal young males as well as in hyperplastic prostatic tissue, obtained from older male patients at the time of surgery, to determine whether an increase in the content of androgen receptors occurs in human BPH in association with increase dihydrotestosterone levels.

Well controlled studies are needed to determine the relationship between systemic endocrinologic changes and the resulting pathology of the prostate.

Vesicoureteral Reflux

Because of the controversies about management of children with vesicoureteral reflux, a multi-center prospective study recently has been funded by the NIADDK. This clinical trial is in the stage of patient recruitment and pilot collection of clinical data. This controlled randomized study, of which the Albert Einstein Medical College is the lead center, involves 18 pediatric centers throughout the country. The aims of the study are to establish a rational basis for the treatment of vesicoureteral reflux so that the development and progression of renal damage in children, can be arrested or halted.

Urolithiasis

Infection-induced urinary stones are manifested clinically as soft, poorly mineralized concretions, or as branched renal calculi that completely fill the upper urinary excretory tract. A collaborative double-blind randomized study is at present being conducted at Baylor College of Medicine to assess the efficacy and safety of the treatment of infection-induced stones with acetohydroxamic acid (AHA). Candidates for the study are patients with resistant urinary infection caused by urea-splitting bacteria and/or infected renal calculi. This study of AHA, an inhibitor of bacterial urease, is in too early a phase to assess the ultimate outcome.

Specialized Centers of Research in Urolithiasis (SCOR's)

In September 1977, five Specialized Centers of Research (SCORS) which have been converted to Program Projects, were funded by NIADDK to conduct research in urolithiasis. Throughout almost four years of support, the impact that the SCOR's in urolithiasis have had in the field of urolithiasis as well as in the overall field of mineral metabolism appears to be great. To assess the impact of the SCOR's (P01) in urolithiasis an evaluation project funded with HHS set-aside evaluation funds will be started in the course of this fiscal year. The applications responding to the Request For Proposals are being evaluated at present.

At the University of Texas Health Sciences Center a reliable ambulatory evaluation protocol was developed. A simply designed thorough outpatient work-up for stone-forming patients is being evaluated. The underlying mechanism leading to kidney stone formation has been determined in most cases. The protocol includes collection of two 24 hour urine samples, one while patients are on a random diet and another, one week after patients have been on a calcium and sodium restricted diet. This period is followed by an overnight fast and two hours of calcium loading. With this simple ambulatory protocol, 241 patients with nephrolithiasis had been evaluated at this center and definitive diagnoses were obtained in 95% of the patients. In most patients, long-term hospital admissions to conduct mineral balance studies, are no longer needed.

Under the same Program, "optimum" treatment modalities are being selected for different forms of nephrolithiasis on the basis of their ability to correct underlying physicochemical and physiological derangements. These treatment modalities include: sodium cellulose phosphate for the absorptive hypercalciurias* (GI tract hyperabsorption of calcium); thiazides for renal (calcium leak) and absorptive hypercalciurias; orthophosphate for hypophosphatemic GI tract absorptive hypercalciurias; allopurinol for hyperuricosuric** calcium oxalate nephrolithiasis--plus thiazide for absorptive hypercalciurias with hyperuricosuria. High fluid intake is recommended for all cases and low calcium diet only for normocalciuric# nephrolithiasis. All seven "optimum" treatment modalities led to significant clinical improvement, and remission or reduction of stone formation as compared with the pretreatment programs. The safety and efficacy of these and other treatment modalities is at present under evaluation.

In the SCOR, at Michael Reese Hospital and Medical Center in Chicago, 850 stone formers were evaluated. Hypercalciuria appeared as an important risk factor and was found in more than 40% of this patient population. Clinical data on these patients is being analyzed so that factors influencing the risk of calculi recurrence in treated patients may be prospectively identified.

In the Chicago Progam, as well as in the program at the University of Florida, Gainesville Campus, efforts are underway to clarify the physicochemical features of calcium oxalate precipitation and aggregation in order to increase the understanding of urinary factors affecting stone formation. Studies to quantify urinary crystallization activity (conversion of stone solutes from solution to solid phase in the urinary tract) in relation to urolithiasis are ongoing. These studies complement the assessment of patient risk of calculogenesis since urinary super saturation and/or crystalluria are found commonly in stone-formers and in normal humans. The critical inducing factors necessary to convert a normal human to a stone-former are being sought. A useful animal model utilizing a catheterized rat bladder which operates as an urothelial-lined living test tube has been developed.

Efforts to characterize the effect of macro and of micromolecules upon the solubility of inhibitors and promoters of crystalization are continuing as well as those to isolate and to characterize inhibitors of crystal growth in the urinary tract i.e., citrate, magnesium, pyrophosphate and several others in different combinations are underway.

Further elucidation of all processes involved in the biological path of urinary stone formation is needed as well as techniques by which this pathway may be altered. Until prevention of urolithiasis is possible, the most appropriate therapeutic management needs to be refined and/or developed.

---

*Hypercalciuria: higher than normal urinary calcium excretion.

** Hyperuricosuria: increased urinanry uric acid excretion.

#: Normocalciuria: normal urinary calcium excretion.

CHRONIC RENAL DISEASE PROGRAM

Introduction

The Chronic Renal Disease Program supports research in maintenance therapies for end stage kidney disease, including hemodialysis, peritoneal dialysis and complications of these therapies. The program also funds research in renal transplantation and organ preservations. Studies concerning the pathophysiology of chronic renal disease including systemic abnormalities such as bone diseases and cardiovascular dysfunctions also constitute a significant segment of the program. Following the brief discussion of developments in kidney transplantation and artificial kidneys, a selection of research highlights from the recent accomplishments of grantees are described.

Developments in Kidney Transplantation and Artificial Kidneys

The main technique used in treatment of chronic renal failure for more than twenty years has been maintenance dialysis (primarily hemodialysis, and also peritoneal dialysis). In 1980 dialysis was provided for about 49,000 patients under the Medicare End-Stage Renal Disease (ESRD) Program while 4700 transplants were performed.

The NIADDK is one of the several institutes of the National Institutes of Health which supports research on transplantation. The NIADDK sponsors studies of organ preservation, techniques of surgery, immunosuppressive therapy, complications of renal transplantation, and transplant immunology. Transplantation is an effective means for treatment of patients with ESRD when well-matched related donor kidneys are available, but when only cadaver kidneys are obtained it is less effective. The requirement for long-term immunosuppressive therapy because of the potential for renal graft rejection and the high incidence of graft failure has resulted in less consistent results than those achieved by ESRD treatment with dialysis. Overcoming the problem of rejection of grafted kidneys continues to be one of the most serious obstacles to a predictably favorable outcome with renal transplantation,although there has been some progress in this area in recent years. In general, patients who receive well-matched organs from closely related donors have the best chance of success among recipients of renal transplants and of a consequent return to a more normal existence.

Many complications arise during the course of treatment of patients by maintenance dialysis. Chronic renal failure and its accompanying "uremia" is a multi-organ system disorder. Problems arise secondary to dialysis treatment and, also regardless of treatment, in relation to the uremic state. Examples of these complications include: neuropathy, primarily impaired sensation and/or impaired muscle strength in limbs; renal osteodystrophy; increased susceptibility to infection, central nervous system symptoms, cardiovascular complications, anemia, and sexual dysfunction. In addition, the well-being of patients on hemodialysis is impaired by occasional muscle cramps, hypotension and post-dialysis fatigue, especially when large amounts of fluids have been removed during dialysis treatments, which usually are given three times each week. The mortality rate among maintenance dialysis patients is about nine percent a year. This figure must be viewed in contrast with certain 100 percent mortality for patients with non-functional kidneys if dialysis or transplantation were not available for treatment.

Improved Organ Preservation

Investigators at the Deseret Foundation, Salt Lake City, Utah have found that substitution of heavy water, D_2O, for normal water offers a means of slowing down catabolic cellular destruction during organ preservation. Dr. Harrison Lazarus and his associates have been studying the effects of organ storage on renal RNA metabolism. Dr. Lazarus has found that after 48 hours of cold storage in D_2O, the free polymerase remaining compared to control was 117% for storage in D_2O compared to 12% in normal water. For bound RNA polymerase, the values were 122% for heavy water compared to 100% for normal water. For one hour of warm ischemia, the free polymerase with heavy water was 92% of control compared to 53% with normal water. The corresponding background values were 85% for heavy water compared to 52% for normal water. These investigators also found that substitution of heavy water for normal water made saline solution as effective as "Sacks" solution for preservation of chromatin function after one hour at 37°C. Dr. Lazarus has begun the study of the substitution of heavy water for normal water in kidneys preserved for 18 hours in saline at 0°C before transplantation. Preliminary data shows that there is a doubling of the amount of ATP and total adenine nucleotides in the kidney with heavy water, 24 hours after transplantation has been performed. Of 20 transplants performed after heavy water-saline, 14 kidneys were functioning whereas, of 20 performed after normal water-saline, 7 kidneys were working. Dr. Lazarus plans further investigation of the possible improvements offered by the substitution of heavy water for practical kidney preservation.

H.M. Lazarus, et al, Summary Progress Report on Grant AM 28139-02, "Effects of Organ Storage on Renal RNA Metabolism," dated February 13, 1981.

The Influence of Cytomegalovirus as a Risk Factor in Renal Transplantation

Investigators at the University of Minnesota have completed a three year prospective study of factors associated with cytomegalovirus (CMV) disease and its influence on the outcome of kidney transplantation. Drs. John Najarian, David Fryd, and associates studied 206 patients, of whom 29% developed overt CMV disease, and 90% of them contracted CMV within the first three months, post-transplant. The effect of the development of overt CMV disease on graft and patient survival is highly significant. The first year graft function rates of those who did not develop overt CMV disease was 79% while 1 year graft function of those who had clinical CMV illness was only 53%. Corresponding one year patient survival rates are 92% and 76%. It is apparent that CMV disease markedly influences outcome for renal transplant patients. A number of risk factors were evaluated by Dr. Najarian and his associates. These included: sex, presence of diabetes, donor type, age, and donor and recipient CMV complement-fixing-titers. The latter is a measure of the prior presence of the disease in an individual. Those factors which were not significant in development of CMV illness were: sex, presence of diabetes, age, and the recipients antibody titer. Factors which were of significance were the origin of the kidney, and the antibody titer of the donor. Patients receiving kidneys from siblings or from a child had a lower probability of developing CMV illness than those who received kidneys from a parent or a cadaveric donor. The most important factor in development of CMV disease and the severity of the resulting disease was the antibody titer of the donor. The investigators note that the incidence of overt disease could be reduced markedly by transplanting kidneys only from CMV negative donors. Unfortunately, approximately one half the kidneys available for transplantation in the two year study were obtained from donors with positive titers. Rather than excluding positive donor kidneys from transplantation, the authors conclude that when a cadaveric positive kidney is available, it should be transplanted into an antibody positive recipient from the waiting list. Other operating policies for the practical management of a kidney transplantation program with respect to control of CMV disease were derived by the investigators.

David S. Fryd, et al. "Cytomegalovirus as a Risk Factor in Renal Transplantation," Transplantation, 30:436-39, 1980.

Improved Kidney Preservation Through Buffer Control

Investigators at the University of California, San Diego, have studied the effects of adjustments of relative acidity of the perfusing solution upon kidney graft survival. Dr. Nicholas A. Halasz and his colleagues have noted that as an aqueous medium is cooled from 37^{o} to 7^{o} C, the pH has to be raised from the essentially biological neutral pH of 7.4 up to a pH of 7.9 in order to maintain the same hydrogen ion concentration. These investigators conducted a set of experiments using dog and rabbit kidneys to determine the effect of various hydrogen ion concentrations upon survival of the animal kidney. Dog kidneys were perfused for a 72 hour period and then reimplanted into the original donor. Rabbit kidneys were perfused for 24 hours prior to testing by an artifical preparation. Dr. Halasz's experimental results demonstrate that there is a plateau in pH between approximately 7.45 and 7.75 at which the best outcomes are reached. At either end of this range, further deterioration of the animal kidney occured. Conventional practice in preserving human kidneys for renal transplantation is to utilize a pH of 7.4. Results of the studies conducted by Dr. Halasz demonstrate that this pH is dangerously close to one at which the preserved kidney would be exposed to too great an acidic environment. The recommendation of the San Diego investigators is that kidneys should be perfused at a higher pH than is common practice, namely at pH 7.6 - 7.65.

John N. Carter, et al, "Studies of the Ideal Hydrogen Ion Concentration for Perfusional Preservation," Transplantation, 30:409-10, 1980.

Structure of a Middle Molecule Uremic Toxin Determined

Drs. Jonas Bergstrom, Horst Jornvall and co-workers at Karolinska Institute, Stockholm, have determined the chemical structure of an abnormal metabolite which accumulates in body fluids of patients with uremia. This compound is a representative of the "middle molecule" group of toxic materials, which have been of strong interest to clinical nephrologists dealing with maintenance dialysis since the early 1970s. The material whose structure has been determined is the primary compound in a middle molecule fraction, referred to as a peak 7C in earlier publications of this investigator. The presence of this peak has been shown to correlate with a number of adverse symptoms in patients with marked uremia. The compound in question has been isolated by an elaborate separation procedure. Structural studies show that the material contains 3 functional groups: an acidic moiety sensitive to removal by acid hydrolysis; glycine in an oligopeptide linkage; and a remaining intermediate-sized acyl group. Identification of various other higher molecular weight abnormal metabolites characteristic of uremia may permit the development of more rational maintenance therapies for these patients. Availability of information on the structures may assist investigators in delineating the causes of production of abnormal metabolites by uremic patients. The present work is a significant step in achieving a better understanding for the management of uremic patients.

L. Zimmerman, P. Fürst, J. Bergström, and H. Jornvall, "A New Gylcine Containing Compound with a Blocked Amino Group from Uremic Body Fluids," Clinical Nephrology, 14, 107-111, 1980.

Racial Differences in Lipoproteins and Accelerated Atherosclerosis in Hemodialysis Patients

In studies of mortality, Dr. Andrew Goldberg and his colleagues at Washington University School of Medicine at St. Louis have observed that mortality due to atherosclerotic heart diseases (ASHD) is 25% among white patients compared to 8% in their black patients. Because hypertension is more prevalent in black patients, the above observation about ASHD is of special note. Measurements of triglycerides, cholesterol, HDL cholesterol, and apoproteins were made. The data showed that white patients have high triglycerides; and reduced high density lipoprotein cholesterols, and apoprotein concentrations. Abnormalities in "atherogenic" lipoprotein occurred more frequently in caucasian dialysis patients than in black dialysis patients. These abnormalities may be related causally to the higher mortality from atherosclerotic heart disease of white population.

Andrew Goldberg, et al, "Racial Differences in Lipoprpteins and Accelerated Atherosclerosis in Hemodialysis Patients," abstract in Clinical Research, Volume 29, Number 2, page 464A, 1981.

Circulating Immune Complexes in Renal Allograft Reactions

Drs. Stanley Jordan, Richard Fine and associates University of Southern California have studied the relationship of high levels of detectible circulating immune complexes and acute rejection episodes of renal allografts in pediatric patients. These investigators studied 27 pediatric renal allograft recipients over a 5 month observation period. During this period, recipients had a mean of 4.5 acute rejection episodes. In 19 recipients who were negative for circulating immune complexes, biopsy of the allograft was performed in 7 individuals and the pathologic interpretation was consistent with acute rejection in all 7. For patients who were positive with respect to tests for circulating immune complexes, biopsy material was available for 3 and studies showed no correlation with levels of circulating immune complexes. These results show no significant relationship between the development of circulating immune complexes and acute rejection. In his study, only 2 recipients showed a correlation of circulating immune complexes with acute rejection. No significant correlation could be established between circulating immune complex generation and allograft outcome.

S. C. Jordan, et al, "Circulating Immune Complexes in Pediatric Renal Allograft Rejection," Transplantation Volume 31, Number 3, 1981, Pages 190-194.

Effect of Chronic Renal Failure on Metabolism of Intact Parathyroid Hormone

The plasma disappearance rate of intact parathryoid hormone (PTH), and the effect of chronic reanl failure on its disappearance rate in the dog were studied by investigators at Washington University, St. Louis. PTH is a critical element in the control of mineral metabolism, and ultimately in potential development of bone disease in renal failure patients. Dr. Hruska and his associates found that the liver and kidney are responsible for all but a small fraction of the metabolic degradation of intact parathyroid hormone. The clearance rate in normal dogs was found to be very rapid and occurred primarily in the liver (61%) and the kidney (31%). The study demonstrated that renal uptake was significantly greater than the fraction of hormone being filtered by the kidney. This data indicates that PTH was being acted upon at sites independent of glomerular filtration. In the chronic renal failure dog model, Dr. Hruska found that the metabolic clearance rate of PTH was reduced to approximately one-half the value of that in the normal animal. Both the hepatic and renal clearance rates were reduced. The liver was found to be the major extrarenal site of PTH metabolism affected by chronic renal failure. Hepatic clearance was reduced by approximately 38% in the chronic renal failure model.

K. A. Hruska, et al, "Peripheral Metabolism of Intact Parathyroid Hormone", Journal of Clinical Investigation, Volume 67, March, 1981, pages 885-892.

OFFICE OF PROGRAM PLANNING AND EVALUATION

The Office of Program Planning and Evaluation (OPPE) has the responsibility for the planning, evaluation and analysis of the Institute's policies, program and research accomplishments. OPPE serves as the focal point for preparation of Congressional testimony and analysis of legislative matters. The Office provides advice to the Institute Director and his immediate staff concerning the implications of pending health legislation and of scientific and policy-relevant reports.

Planning

The OPPE guides the Institute in a continual cycle of planning activities. Each year, the Office distributes guidance for the fall intra-Institute Director's review; this guidance was revised completely for Fiscal Year 1981. In response to this guidance, the staff of the four extramural program clusters and the Office of the Associate Director for Extramural Affairs each develops a program plan with a summary of program accomplishments and research advances, research opportunities, updated research initiatives, and multi-year budget projections for research and training. These plans are reviewed by the Director and other Institute officials and then discussed in a series of program reviews, usually held in November and December. These reviews help prepare the Director for testifying before the Congressional Appropriations Committees and for discussing Institute plans with the Director, NIH. The Office helps program staff to prepare these oral and written presentations.

Based upon these documents, OPPE writes the Institute's annual Research Plan. The Office also develops briefing materials for use by Institute management during the NIH Director's annual review of NIADDK programs and budget. This year, OPPE staff were responsible for preparing contributions to a wide variety of NIH and departmental reports and plans.

Three planning activities were new to the Office in FY 1981. For the first time, the Institute was asked to make a presentation to the Director, NIH, on an issue of long range planning. The Institute chose to discuss the plans and initiatives of its Diabetes Program. The Office was instrumental in preparing for this presentation.

Following passage of P.L. 96-538, the Health Programs Extension Act of 1980, OPPE staff assisted in developing a plan for implementing this legislation. This Act renamed the Institute, extended numerous Institute programs, authorized establishment of three Boards--in arthritis, diabetes, and digestive diseases--(the first two are being reestablished, the latter is new), mandated organizational positions and entities already established administratively, and instituted new reporting requirements. As one example, the law requires the Director, NIADDK, to submit an annual report to Congress and the President on or before November 30 describing the Institute's activities in arthritis, diabetes, and digestive diseases.

Before this report was mandated, the Office had initiated a project to publish an annual report of the Director including the Institute's program on kidney diseases, as well as all other programs designated in the Act. The Fiscal Year 1981 Director's Report will be the first report of this kind. This report will summarize the accomplishments of the Institute over the 30 plus years of its existence and will outline the program activities and plans of NIADDK.

Program Analysis

OPPE staff continually receive and respond to many program analysis requests. A response to these requests typically require a review of the entire Institute's research programs in order to determine Institute involvement in the subject area or disease in question. Responses generally include explanatory narrative material, itemized listing of funds expended and/or projected, and a listing of grants and intramural projects being conducted relevant to the subject of the request. The following are examples of program analyses performed by OPPE staff during fiscal year 1981:

- Population Research
- Alcohol and Drug Abuse Research
- Technology Assessment and Transfer
- Identification of Emerging Health Technologies
- Immunosuppressive Techniques in Renal Transplantation
- Analysis of the Portfolio on Trauma and Burn Support
- Synthesis of Hormones and Related Polypeptides for Pharmacotherapy
- Use of live animals in research and testing

In addition, OPPE staff supply substantial analytical input into a number of documents, including the following:

- NIH Annual Report on International Activities
- Primary Prevention
- International Nutrition Research and Training Activities
- Interagency Surveys of Government Exchange Programs

Reports

OPPE is the central point for the preparation of various ad hoc reports and the review, clearance, and official transmittal of other reports to the Secretary and/or the Congress. A number of reports were prepared during fiscal year 1981 concerning medical advances of the Institute in the last decade; Institute involvement in research on aging, Reye's Syndrome, nutrition, alcoholism and drug abuse, intramural projects related to diabetes; and on various other specifically requested subject matter.

Reports were prepared for the House Committee on Appropriations for Labor and Health and Human Services regarding the report "Research Needs and Priorities in Dermatology" and on the status of implementation of the recommendations of the National Diabetes Commission. The Fourth Annual Report on Evaluation of Multipurpose Arthritis Centers was submitted to Congress in January 1981.

The OPPE, in conjunction with a contractor, is currently preparing the first Annual Report of the NIADDK Director. This report will contain retrospective, current, and prospective data on programmatic activities and resources, and thus, will serve as a highly useful compendium of information for Institute staff, the biomedical research community, voluntary health organizations, the Congress, and other individuals and groups having a particular interest in NIADDK.

Evaluation

The OPPE prepares the Institute's Annual Evaluation Plan which consists of background information; evaluation strategy of the Institute; and a description of new proposed evaluation projects, ongoing projects and recently completed projects. The OPPE collaborates with the extramural clusters in preparing this plan, providing advice on criteria for evaluation projects, monitoring ongoing projects and preparing the necessary submissions for completed projects.

Evaluation projects recently completed include an "Evaluation of Research Alternatives to Minimize SSA Medicare Costs for Dialysis Treatments, and an "Evaluation of the Hematology Program of NIADDK in Relation to Identification of Research Needs."

The Institute is initiating several new evaluation projects during fiscal year 1981. These projects include developing methodology for the evaluation of NIADDK Center Grant Programs; the evaluation of the Urolithiasis Centers, the non-research activities of the multipurpose arthritis centers, and the Musculoskeletal Diseases Program.

Impact Statements were submitted on five evaluation projects that have been completed in the past four years. These statements include a description of the program involved, the study design, recommendations resulting from the study, organization response to the recommendations, the actual changes adopted and the implementation process for those changes, and the effects of any programmatic changes. The Impact Statements will be utilized by the Department for inclusion in a report on the utilization of evaluation project results.

Legislation

Title II of the Health Programs Extension Act of 1980 (P.L. 96-538) redesignated NIAMDD as the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases and the title of its Advisory Council was changed accordingly. The position of Associate Director for Kidney, Urologic, and Hematologic Diseases was established while the authority for the three other Associate Directorships was continued. Statutory NIH-wide efforts were established for each Associate Director with respect to the diseases within their purview. In addition, the bill stipulated changes in reporting requirements, Advisory Council functions, and numerous amendments affecting the organization and operations of the Institute.

OPPE staff performed detailed analyses of pending legislation pertinent to the Institute; attended Congressional hearings; prepared legislative briefing materials for use by Institute management; coordinated the preparation of answers to senate and house hearings questions; developed NIADDK legislative proposals; and prepared a number of bill reports outlining the Institute's position with respect to legislation relevant to NIADDK.

Medical Applications of Research

As NIADDK's point of official liaison with the NIH Office of Medical Applications of Research (OMAR), the OPPE is responsible for recommending appropriate areas for consensus development conferences; reporting on emerging technologies identified by scientific staff as having the potential for impacting on medical practice; and representing the Institute at OMAR advisory committee meetings and conferences.

During fiscal year 1981, OPPE staff also responded to frequent requests from OMAR to review the state-of-the-science with respect to various drugs, devices, and techniques whose eligibility for coverage through Medicare was under active consideration by the Health Care Finance Administration, Medicare coverage issues addressed by OPPE included the following:

- Anti-Lymphocytic Globulin and Anti-Thymic Globulin as an Immunosuppresive Technique in Renal Transplantation
- Carbon Dioxide Laser Surgery
- Various Tests and Procedures for Arthritis
- Hyperbaric Oxygen Therapy for Treatment of Chronic Refractory Osteomyelitis and Arthritis
- Plasmapheresis in the Treatment of Rheumatoid Arthritis
- Electromagnetic Treatment of Bone Fractures
- Rehfuss Test/Orthomolecular Medication and Megavitamin Therapy/Intragastric Hypothermia Using Gastric Freezing
- Blood Seromucoid Test
- Treatment of Obesity
- Photodensitometry, Bone Histology, and Absorptometric Procedures for Bone Mineral Analysis
- Home Penile Tumescense Monitoring
- Blood Glucose Analyzers for Use in the Patient's Home
- Breath Hydrogen Lactose Tolerance Test for Diagnosing Lactose Intolerance
- Lactulose Breath Hydrogen Test
- $^{13}CO_2$ Breath Test Diagnosing Fat Maldigestion and Malabsorption
- Glucose Controlled Insulin Infusion System
- Shortwave Diathermy

Review

Throughout the year, OPPE staff have supplied critical comments on a variety of documents referred to the Institute for review. These included:

- GAO Draft Report: Research Planning and Evaluation at the NIH and Aspects of Advisory Council Operations
- FY 80 International Nutrition Research and/or Training Activities
- Thirty-fourth World Health Assembly-Agenda Comments
- Review of NCHCT Recommendations to Medicare on Reimbursement of Plasmapheresis in the Treatment of Rheumatoid Arthritis

ANNUAL REPORT SUMMARY

Epidemiology and Field Studies Branch

The research activities of the Branch include epidemiologic studies of diabetes, digestive diseases, arthritis and other metabolic diseases. In addition, the Branch continues to provide epidemiological and biostatistical support to other components of the Intramural and Extramural programs of the Institute. The major focus of the Branch continue to be the investigation of the distribution and pathogenesis of diabetes and obesity prevailing in the inhabitants of the Gila River Indian Reservation.

For the past year Dr. Peter Bennett has been on assignment with the South Pacific Commission in New Caledonia conducting epidemiologic studies of diseases endemic to that area of the South Pacific.

Southwestern Field Studies Section

The Southwestern Field Studies Section has continued to investigate the determinants of diabetes and its complications primarily from the ongoing prospective study of the natural history of diabetes in the Pima Indian population. Investigation of genetic determinants of diabetes in the Pima Indian population has recently concentrated on the relationship of diabetes to HLA antigens. The observation of an association between HLA-A2 and diabetes in elderly Pimas has been extended with the typing of younger subjects. The association was found in all age groups studied. HLA-A2 phenotype frequencies were inversely associated with age in the diabetic subjects, suggesting that HLA-A2 is in linkage disequilibrium with a diabetes - susceptibility gene which contributes to an earlier age of onset of diabetes.

Studies of the causes and development of diabetes have continued in collaboration with the Phoenix Clinical Research Section. Diabetes in the Pimas is Type II, in that it is not insulin dependent, not associated with islet-cell antibodies or HLA antigens typical of Type I diabetes, and Pima diabetics retain basal insulin secretion and secrete insulin after intravenous arginine stimulation. Because of their genetic isolation, there is a greater chance that a single genetic mechanism is involved in diabetes in the Pimas. Obesity is extremely common, with 92% of Pima men and 94% of the women 20-24 years old exceeding the U.S. medians of body mass index (BMI) for their age. Obesity and parental diabetes have a marked effect on diabetes incidence, and they interact strongly. Obese Pimas with diabetic parents have the highest diabetes incidence. Pimas are resistant to insulin-mediated glucose disposal. However, even diabetic Pimas with marked fasting hyperglycemia are very sensitive to the antilipolytic action of insulin. These differences in insulin sensitivity may facilitate the development of obesity, and may have allowed the Pimas and other similar populations to survive famines. When food supplies of such people become abundant and constant, the development of sustained obesity may lead to impaired glucose tolerance, hypersection of insulin, eventual beta cell failure, and diabetes. Thus Neel's "thrifty genotype" may be mediated through selective insulin resistance.

In collaboration with the Indian Health Service Hospital, Keams Canyon Arizona, studies of the natural history of microvascular complications of diabetes were extended. Navajo and Hopi Indian patients with diabetes mellitus were examined for retinopathy and proteinuria using methods similar to those of the study of Pima in the Gila River Indian Community. Diabetes duration-specific prevalence rates of retinopathy and proteinuria were very similar in Hopis, Navajos, and Pimas, although all three tribes are culturally and linguistically distinct. These findings emphasize the universal natural of microvascular complications of diabetes. Diabetic retinopathy is strongly dependent on the duration of diabetes but appears not to depend on racial or geographic characteristics.

Data from the prospective study of glucose tolerance and its relationship to pregnancy outcome in the Gila River Indian population has resulted in quantification of the risks associated with various degrees of glucose intolerance to pregnancy outcome. The effects of maternal diabetes on the development of obesity in 5-14 year old offspring of Pima Indian women were investigated. Offspring of mothers who were diabetic during pregnancy were more obese than offspring of mothers who were either nondiabetic (through out all followup) or prediabetic (i.e. first became diabetic after the delivery). This suggests that the maternal environment during gestation has a subsequent effect on obesity in the offspring. Obesity, itself a strong risk factor for diabetes, is more frequent, more severe and develops earlier in the offspring from diabetic than from prediabetic or nondiabetic pregancies. The prenatal environment may affect weight not only at birth, but later in childhood and in the teenage years.

Familial aggregation of clinical gallstone disease was studied in an attempt to find evidence for genetic determinants for this common disease of Pima Indians. The frequency distribution of affected siblings in families was very close to expectation under a random distribution of the disease in the population, but was incompatible with a simple dominant or recessive mode of inheritance. Thus if clinically detected cholelithiasis is an inherited disease in the Pimas, most Pimas may have the necessary gene(s), with the major factors distinguishing Pimas with and without gallstones being non-familial.

The Southwestern Field Studies Section has continued studies in the etiology of rheumatoid arthritis. In patients with rheumatoid arthritis and in age-sex matched controls, titers of Epstein-Barr virus nuclear antigens (EBNA) were not related to the disease. The titers of EBNA in the Pima sample as a whole were very high, suggesting chornic antigenic stimulation. The findings suggest that Epstein-Barr virus infection is not involved in the pathogenesis of rheumatoid arthritis in Pimas. Rheumatoid factor was examined as a risk factor for later development of rheumatoid arthritis. The incidence of rheumatoid arthritis increased from 1.61 cases/1000 person-years in subjects with rheumatoid factor titers of 1:4 or less to 6.16 cases/1000 person-years in subjects with rheumatoid factors of at least 1:64. Thus, although the causes of the high titers of rheumatoid factor in this population remain unknown, rheumatoid factor has an important predictive, and perhaps causal, role in the disease which warrants further investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 69000-16 EFS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Diabetes Mellitus and Other Chronic Diseases in the Gila River Indian Community

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.H. Bennett	Chief	EFSB, NIAMDD
Other:	W.C. Knowler	Chief	SFSS, NIAMDD
	D.J. Pettit	Asst. Chief	SFSS, NIAMDD
	J.R. Lisse	Staff Physician	SFSS, NIAMDD
	E.J. Ballintine	Clinical Director	IRP, NEI
	D.L. Mann	Senior Investigator	IB, NCI

COOPERATING UNITS (if any)

N.B. Rushforth, Case Western Reserve University; R.C. Williams, Arizona State University; R. Rate, Indian Health Service Hospital, Keams Canyon, Arizona

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.9	5.5	5.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The natural history of the various manifestations and vascular complications of diabetes mellitus and other chronic diseases are being studied and evaluated in an Amerindian community. Various associated conditions and factors, including heredity, body weight, and blood pressure, that may have a bearing on the frequency and severity of diabetes and/or its complications are under investigation.

Project Description:

Objectives:

1. Determine the prevalence and incidence of diabetes mellitus among those five years of age and over on the Gila River Indian Reservation.
2. Evaluate factors associated with the progression from the prediabetic state to subclinical diabetes mellitus and to clinical diabetes.
3. Study risk factors associated with the rate of appearance and progression of vascular complications.
4. Investigate influence of tribe, age, obesity, parity and other factors on changes in glucose tolerance and their relationship to diabetes mellitus.
5. Determine the mode of inheritance of diabetes in the Pima Indians.

Methods Employed:

1. The following tests and examinations are repeated at two yearly intervals on all Indians five years of age or more living on the Sacaton Service Unit portion of the Gila River Indian Reservation.

   a. Clinical interview
   b. Physical examination
   c. Modified glucose tolerance test and other biochemical tests

   In addition, on those aged 15 years and over:

   d. Fundus photographs
   e. Electrocardiogram
   f. Tests for vibratory sensation threshold
   g. Soft tissue radiographs for vascular calcification

2. Some of the above tests and examinations are also being done on Indians of other tribes or living under different environmental conditions and on non-Indian subjects for comparison.

3. Blood typing is performed on all subjects for red cell antigens, serum proteins, and Gm antigens. These typings provide an extensive set of approximately 40 gene markers for use in genetic analysis. In addition, selected individuals are HLA typed.

4. Medical records (inpatient and outpatient) are reviewed at each biennial examination. Diagnoses of diabetes, cholelithiasis or cholecystitis, and other chronic diseases are noted.

Major Findings:

1. Association of HLA-A2 Antigen with Diabetes. HLA antigens have been associated with type I diabetes mellitus in many populations, but only rarely with type II diabetes mellitus. We determined the relationship between HLA antigens and diabetes (2 hr. post load plasma

glucose concentration $\geq$200 mg/dl) in the Pima Indians of Arizona, a population with a very high incidence of diabetes, which is almost entirely type II (non-insulin dependent). HLA typing was done by standard microcytotoxicity techniques. HLA-A locus antigens were determined in 371 full-blooded Pima Indians, none of whom were first degree relatives. Full-blooded Pimas have a limited distribution of HLA antigens and little evidence of admixture from other populations. HLA-A2 was found in 81% of the 229 diabetics and 68% of the 142 non-diabetics (relative risk, RR,=2.2, $p<.005$). The association of HLA-A2 and diabetes remained when subjects were stratified by age (adjusting for age in 3 groups, summary RR=2.2, $p<.005$). In the diabetics, but not in the entire study group, homozygosity for HLA-A2 was inversely related to age, with 60%, 33%, and 29% of diabetic subjects <35, 35-54, and $\geq$55 years old, respectively, being homozygous for HLA-A2 ($p<.05$ for the association of homozygosity with age). The HLA-A2 phenotype frequencies were 95%, 82%, and 79% in the diabetics <35, 35-54, and $\geq$55 years old, suggesting that HLA-A2 is in linkage disequilibrium with a diabetes-susceptibility gene which, in a dose-related fashion, contributes to an earlier age of onset of diabetes.

2. Obesity, Insulin Resistance, and Diabetes Mellitus in the Pima Indians. Diabetes mellitus has long been regarded as a disease with a strong hereditary basis, but understanding its genetics has been hampered by problems in diagnosing and classifying the disease. Onset of diabetes in adulthood makes many individuals with a diabetes genotype impossible to identify during much of their lives. A further problem is that diabetes may be heterogeneous in terms of etiology. No single mode of inheritance is likely to be involved in all types of diabetes in all populations. Diabetes genetics is also complicated by obesity, a strong risk factor for type II diabetes. Obesity might be either an environmentally induced factor affecting the expression of a diabetes genotype, or the initial manifestation of a genetically determined diabetes-obesity syndrome. Neel has hypothesized that the introduction of a steady food supply to people who have evolved a "thrifty genotype" leads to obesity, insulin resistance, and diabetes. This genotype may have had selective advantage by allowing efficient energy storage during times of plenty thus permitting survival during famine.

   For about 2000 years, the ancestors of the Pima Indians of the southwestern United States are thought to have lived in isolation in the desert with alternating periods of feast and famine. Obesity and diabetes have become extremely common only recently, coincident with disruption of the traditional lifestyle and introduction of an abundant food supply. The Pimas now participate in an epidemiologic study including periodic measurement of obesity and glucose tolerance before and after the onset of diabetes, thus allowing classification of the members of this defined population.

Today the Pimas have a high prevalence of diabetes, the development of which is familial and strongly related to obesity. Fifty percent of the Pimas at least 35 years old have diabetes, defined by a 2 hr. post-load plasma glucose concentration of at least 200 mg/dl (11.1 mmol/l). Diabetes in the Pimas is type II, in that it is not insulin dependent, not associated with islet-cell antibodies or HLA antigens typical of type I diabetes, and Pima diabetics retain basal insulin secretion and secrete insulin after intravenous arginine stimulation. Because of their genetic isolation, there is a greater chance that a single genetic mechanism is involved in diabetes in the Pimas. Obesity is extremely common, with 92% of Pima men and 94% of the women 20-24 years old exceeding the U.S. medians of body mass index (BMI) for their age. Obesity has a marked effect on diabetes incidence, which increases steadily from near zero in those with a BMI of less than 20 kg/m^2 to a rate of 72 new cases/1000 person-years in those with a BMI of at least 40 kg/m^2. Furthermore, obesity and parental diabetes interact strongly. Obese Pimas with diabetic parents have the highest diabetes incidence. Despite this effect, obesity, except in the extreme, does not increase mortality, consistent with the hypothesis that the Pimas evolved the ability to become obese as a survival measure.

Pimas with impaired glucose tolerance or diabetes without fasting hyperglycemia have elevated fasting and stimulated insulin concentrations compared with nondiabetic Pimas with similar degrees of obesity. They are resistant to insulin-mediated glucose disposal. However, even diabetic Pimas with marked fasting hyperglycemia are very sensitive to the antilipolytic action of insulin. These differences in insulin sensitivity may facilitate the development of obesity, and may have allowed the Pimas and other similar populations to survive famines. When food supplies of such people become abundant and constant, the development of sustained obesity may lead to impaired glucose tolerance, hypersecretion of insulin, eventual beta cell failure, and diabetes. Thus, Neel's "thrifty genotype" may be mediated through selective insulin resistance.

3. The Effects of Parental Diabetes on the Relationship of Obesity and the Incidence of Diabetes. The incidence of diabetes mellitus was determined in the Pima Indians of Arizona, during a 15 year longitudinal study that included periodic measurements of weight, height, and glucose tolerance. Diabetes incidence was strongly related to preceding obesity in both sexes and at all ages in people at least 15 years old. In contrast, there was little relationship between diabetes prevalence and concurrent obesity, illustrating the importance of longitudinal studies in estimating the effect of obesity on the occurrence of diabetes. In studying the development of a disease such as diabetes which is manifested by weight loss, care must be taken to consider sequential changes in body weight rather than weight measured only at one point in time. Comparing weights of people after they have developed diabetes with weights of nondiabetics

can be especially misleading. In prevalence studies, weight loss following the development of diabetes could lead to considerable underestimation of the etiologic relationship of obesity and diabetes, as illustrated in the Pimas. Although incidence or longitudinal studies are much more time consuming than prevalence studies, they may be essential for understanding the magnitude of the role of obesity in the development in diabetes.

The association of diabetes incidence with obesity remained within each group when subjects were classified by the diabetes status of their parents, also an important risk factor for diabetes. Parental diabetes enhanced the effect of obesity in increasing diabetes incidence.

In the Pimas, both obesity and diabetes have become more common during the last few decades, probably as a result of rapid cultural and dietary changes in a population genetically susceptible to diabetes. These findings have important implications to many other parts of the world where similar increases in obesity and diabetes appear to be occurring.

4. Comparison of Microvascular Complications of Diabetes in Three Tribes of American Indians. Navajo and Hopi Indian patients with diabetes mellitus were examined for retinopathy and proteinuria using methods similar to those of the study of Pimas in the Gila River Indian Community. Diabetes duration-specific prevalence rates of retinopathy and proteinuria were very similar in Hopis, Navajos, and Pimas, although all three tribes are culturally and linguistically distinct. These findings challenge previous reports by others that hyperglycemia in Hopis and Navajos is an isolated metabolic abnormality unassociated with microvascular complications typical of diabetes. Possible methodologic differences in the previous studies may be responsible for their failure to find many patients with complications. The present study emphasizes the universal nature of microvascular complications of diabetes. Diabetic retinopathy is strongly dependent on the duration of diabetes but appears not to depend on racial or geographic characteristics.

5. Lack of Familial Aggregation of Gallstones. In Pima Indian women, no evidence of familial aggregation of gallbladder disease was found, as assessed by routine clinical means. Medical records were reviewed for cholelithiasis as evidenced by surgical findings or oral cholecystography (performed only when clinically indicated for medical care). Familial aggregation was assessed in 316 Pima women aged 35 through 84 years who belonged to 144 sibships. They represented 116 families with two sisters in this age range and 28 families with three. In these women the disease prevalence was 143/316=45%. There was little variation of prevalence with age within this range. The numbers of families having 0, 1, and 2 sisters with gallstones among families with two sisters, and 0, 1, 2, and 3 affected sisters in families of

three were tabulated. The frequency distribution was very close to expectation under a random distribution of the disease in the population (test for goodness of fit to a random distribution, $X^2 = 3.92$, d.f.=5). However, the distribution was incompatible with a simple dominant or recessive mode of inheritance. Thus if clinically detected cholelithiasis is an inherited disease in the Pimas, most Pimas may have the necessary gene(s), with the major factors distinguishing Pimas with and without gallstones being non-familial.

Significance to Biomedical Research and the Program of the Institute:

Previous findings of an association of type II diabetes with the genetic marker HLA-A2 have been extended. The association and an inverse relation of the frequency of homozygosity for HLA-A2 with age in diabetic subjects suggest that HLA-A2 is in linkage disequilibrium with a diabetes-susceptibility gene which contributes to an earlier age of onset of diabetes. This information, combined with other studies of diabetes etiology described under this and other projects, may help in our understanding the cause(s) of diabetes.

Obesity and parental diabetes are the strongest risk factors yet identified for diabetes in Pima Indians. They interact strongly, with obese offspring of diabetic parents having extremely high diabetes incidence rates. Because of weight loss following the development of diabetes, prevalence (cross-sectional) studies can seriously underestimate the effect of obesity on the development of diabetes. Thus incidence (longitudinal) studies may be essential for understanding the role of obesity in the development of diabetes.

Resistance to insulin mediated glucose disposal is a feature of obese, nondiabetic Pima Indians which is likely to be involved in the development of diabetes. Whether insulin resistance is a primary abnormality in Indians or is secondary to obesity is an important question to be addressed in future research.

A comparison of microvascular complications of diabetes in three tribes of American Indians supports the hypothesis that these complications are primarily a function of diabetes duration and depend little on racial or geographic factors. This is consistent with our previously reported findings that the frequency of microvascular, but not macrovascular, complications was similar in Japanese diabetics living in Japan, diabetics of Japanese descent in Hawaii, and Pima Indians. These findings suggest that research about microvascular complications in the Pima Indians is also relevant to other diabetic people.

Genetic or environmental causes of gallstones remain unknown. The lack of familial aggregation of cholelithiasis suggests either that a simple genetic mechanism does not predominate or that most Pimas have the necessary gene(s), with the major factors distinguishing Pimas with and without gallstones being non-familial.

Proposed Course:

The long term followup studies of diabetes and its vascular complications will continue. Surveillance of non-diabetic subjects becoming diabetic and of diabetics progressing with their disease gives an opportunity for further study or risk factors diabetes and its complications. Further study of risk factors for diabetes will include studies of obesity, HLA type, and other gene markers. We will attempt further characterization of the mode of inheritance of diabetes in Pima Indians by studying segregation of the disease in pedigrees and linkage with HLA and other gene markers. Further studies of the role of blood pressure, plasma glucose concentration, and drug treatment on the incidence of diabetes retinopathy and nephropathy will be undertaken.

Publications:

1. Bennett, P.H., and Savage, P.J.: "Prognostic Factors for the Development of Diabetes Mellitus in Pima Indian Children and Young Adults." in Grave, G. (ed.): Early Detection of Potential Diabetics: The Problems and the Promise, New York: Raven Press (1979), pp. 53-67.

2. Pettitt, D.J., Knowler, W.C., Lisse, J.R., and Bennett, P.H.: Development of retinopathy and proteinuria in relation to plasma glucose concentrations in Pima Indians. The Lancet 2:1050-1052, 1980.

3. Bennion. L.J., and Knowler, W.C.: Epidemiology of gallstones. In: Rotter, J.I., Samloff, I.M., and Rimoin, D.L., eds: The Genetics and Heterogeneity of Common Gastrointestinal Disorders, Academic Press, New York, pp. 297-312, 1980.

4. Bennett, P.H., Knowler, W.C., Pettitt, D.J., and Lisse, J.R.: The prognostic significance of the glucose tolerance test. In: Cumming, I.A., Funder, J.W., and Medelsohn, F.A.O., eds: Endocrinology 1980: Proceedings of the 6th International Congress of Endocrinology, Elsevier/North-Holland, Amsterdam, 1980.

5. Knowler, W.C., Pettitt, D.J., Savage, P.J., and Bennett, P.H.: Diabetes incidence in Pima Indians: contribution of obesity and parental diabetes. American Journal of Epidemiology 113:144-156, 1981.

6. Bennett, P.H.: The Epidemiology of Diabetes. In: Rifkin, H., and Raskin, P., eds: Diabetes Mellitus, Vol. V., Bowie, Maryland, Robert J. Brady Company, pp. 87-94, 1981.

7. Howard, B.V., Lisse, J.R., Knowler, W.C., Davis, M.P., Pettitt, D.J., and Bennett, P.H.: Diabetes and artherosclerosis in Pima Indians. In: Proceedings of the Symposium on Atherosclerosis and Diabetes, New York (in press).

8. Knowler, W.C., Bennett, P.H., Pettitt, D.J., and Savage, P.J.: obesity and diabetes in Pima Indians: the effects of parental diabetes on the relationship of obesity and the incidence of diabetes. In: Proceedings of the Third Symposium on Diabetes Mellitus in Asia and Oceania, Honolulu, Excerpta Medica, Amsterdam (in press).

9. Knowler, W.C., Savage, P.J., Nagulesparan, M., Howard, B.V., Pettitt, D.J., Lisse, J.R., Aronoff, S.L., and Bennett, P.H.: Obesity, insulin resistance, and diabetes mellitus in the Pima Indians. In: Tattersall, R.B., and Köbberling, J., eds: Proceedings of the Second International Conference on the Genetics of Diabetes Mellitus, Freiburg, Germany, Academic Press (in press).

10. Williams, R.C., Knowler, W.C., Butler, W.J., Pettitt, D.J., Lisse, J.R., Bennett, P.H., Mann, D.L., Johnson, A.H., and Terasaki, P.I.: HLA-A2 and type II diabetes mellitus in Pima Indians: an association of allele frequency with age. Diabetologia (in press).

11. Pettitt, D.J., Lisse, J.R., Knowler, W.C., and Bennett, P.H.: Mortality as a function of obesity and diabetes mellitus. American Journal of Epidemiology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69001-12 EFS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Complications and Outcome of Diabetic and Prediabetic Pregnancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	P.H. Bennett	Chief	EFSB, NIAMDD
Other:	W.C. Knowler	Chief	SFSS, NIAMDD
	D.J. Pettitt	Assistant Chief	SFSS, NIAMDD
	J.R. Lisse	Staff Physician	SFSS, NIAMDD

COOPERATING UNITS (if any)

Indian Health Service
Dr. Kirk Aleck, Department of Pediatrics, University of New Mexico

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS: 1.9	PROFESSIONAL: 1.4	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To determine the effects of diabetes mellitus and prediabetes on the complications and outcome of pregnancy in women in the Gila River Indian Community.

The characteristics of all pregnancies among residents of the Gila River Indian Community are determined. Each pregnant woman has a modified glucose tolerance test during the third trimester and following delivery and is retested at approximately two yearly intervals in conjunction with the prospective study of diabetes mellitus in the same community. The characteristics of the pregnancies in women who subsequently become diabetic will be compared to those among women who were diabetic at the time of pregnancy and those who remain nondiabetic. Children from these pregnancies are examined at approximately two yearly intervals after age 5 years for height, weight, and glucose tolerance, in order to determine long-term effects of the characteristics of pregnancies on the offspring.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To document the natural history and to determine the effect of diabetic and prediabetic pregnancies in a Pima Indian group with the highest recorded prevalence of diabetes mellitus. Special emphasis will be placed on complications during pregnancy and associations with mortality and morbidity in the offspring, both in the perinatal period and later in childhood.

Methods Employed: The course and outcome of pregnancies in all female residents of the Gila River Indian Community (Sacaton Service Unit area) are followed and recorded in a standardized manner, and the children are followed from age 5 years on. Data is supplemented by reviews of the Sacaton Hospital charts.

All patients attending prenatal clinic at Sacaton Hospital receive a modified glucose tolerance test during the third trimester of their pregnancy. These patients receive another modified glucose tolerance test 24-48 hours post partum and a chart review and patient interview are done for additional information. They are retested at approximately 2 yearly intervals to document the development of diabetes. They will also be retested during each subsequent pregnancy.

Infants will be examined to determine the presence of congenital anomalies and data related to mortality and morbidity will be collected and documented. At age five years, the children will be re-examined to detect any further abnormalities which may have been inapparent at birth.

Major Findings: The effects of maternal diabetes on the development of obesity in 5-14 year old offspring of Pima Indian women were investigated. Three groups of children were identified according to whether the mother was diabetic, prediabetic, or nondiabetic during pregnancy. The diabetic mothers had 2-hour post-load plasma glucose concentrations (2HPG) of at least 200 mg/dl; the nondiabetic mothers always had a 2HPG of less than 140 mg/dl; and the prediabetic mothers first developed 2HPGs > 200 mg/dl after delivery.

In younger children (5-8 yrs old), 19.9% of offspring of nondiabetics were obese ($\geq$120% ideal weight) compared to 26.3 percent in offspring of prediabetics and 59.1% in offspring of diabetics. The means of body weight were 111%, 115% and 126% of ideal weight respectively. In other children (11-14 years old) the offspring of nondiabetics, prediabetics, and diabetics were 120%, 127% and 156% of ideal body weight respectively and obesity was found in 38.8%, 56.8% and 92.6% respectively.

Obesity, itself a strong risk factor for diabetes, is more frequent, more severe and develops earlier in the offspring from diabetic than from prediabetic or nondiabetic pregnancies. The prenatal environment may affect weight not only at birth, but later in childhood and in the teenage years.

Significance to Biomedical Research and Program of the Institute: In view of the high prevalence of diabetes in the Pima Indians, studies in the Gila River Community provide a unique opportunity to evaluate the outcome of both prediabetic pregnancies and pregnancies with mild carbohydrate intolerance. Retrospective studies of the course of prediabetic pregnancies have to date produced conflicting results. Although it is well established that patients with severe glucose intolerance, especially those with diabetes of long duration and significant microvascular complications, have an increased prevalence of fetal mortality and maternal morbidity, it is not certain whether treatment will improve the outcome of those pregnancies in which there is mild glucose intolerance. Limited prospective studies of the benefit of treating pregnant women with mild glucose intolerance have so far yielded conflicting results. With the adoption of more stringent criteria for the treatment of mild carbohydrate intolerance in pregnancy it is hoped that this study will be able to add to the evaluation of this problem by monitoring the impact of these therapeutic changes on the outcome of pregnancies on the reservation.

Proposed Course: The pregnancy study will continue in conjunction with the study of the natural history of diabetes mellitus in the Gila River Indian Community. The number of pregnancies recognized to be prediabetic will increase as the mothers studied develop diabetes mellitus. In addition, major emphasis will be placed on obtaining followup examination on the offspring of the women in the study.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69006-11 EFS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Gila River Indian Community Autopsy Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	P.H. Bennett	Chief	EFSB, NIAMDD
Other:	W.C. Knowler	Chief	SFSS, NIAMDD
	D.J. Pettitt	Assistant Chief	SFSS, NIAMDD
	J.R. Lisse	Staff Physician	SFSS, NIAMDD

COOPERATING UNITS (if any) D. Kuban, Chief, Pathology Dept., Phoenix Indian Medical Center; P. LeCompte, Clinical Professor Emeritus, Harvard Medical School: L. Klein, Professor of Orthopedic Surgery and Biochemistry; Case Western Reserve Univ.· L. Orci, Chairman, Dept. of Morphology, University of Geneva

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The postmortem characteristics of Pima Indians of the Gila River Indian Community are investigated so that findings in subjects with and without diabetes mellitus can be correlated with studies in living subjects.

Postmortem examinations are performed on members of the Gila River Indian Community whenever possible, regardless of whether the subjects have prior evidence of diabetes. Tissues are examined by light and electron microscopy and the histology related to the clinical findings in the same subjects. The histology of the pancreatic islets of Langerhans is being studied in detail to describe the changes that take place in diabetes. The distribution of cell types varies considerably between different regions of the pancreas suggesting regional variation in function.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

1. To determine the causes of death, and associated disease processes in members of the Gila River Indian Community being followed in prospective studies on arthritis, diabetes mellitus, cholelithiasis, etc.

2. To ascertain and evaluate the histopathological changes in tissues and cells as they relate to diabetes mellitus. To assess their importance and relationship to clinical findings in members of the Gila River Indian Community upon whom autopsies are performed.

3. Evaluate factors and conditions associated with increased or decrease mortality rate in the Gila River Indians.

Major Findings: Accumulation of data on cause of death and histology of tissue continues.

Significance to Biomedical Research and the Program of the Institute: As more subjects who have been under long term ante-mortem surveillance are autopsied, more precise clinical pathologic correlations will be available for the study of diabetes mellitus than has previously been possible.

Proposed Course: The systematic collection and cataloging of tissues will continue. As sufficient specimens become available, detailed examinations of various tissues will be made. Further studies of the kidney, including electron microscopy and biochemical quantification of the insulin, glucagon, and pancreatic polypeptide producing cells of the Islets of Langerhans will be the major activity in the coming year. Data on cause of death will be related to antecedant conditions such as diabetes, obesity, and to genetic markers such as HLA type.

NIH Contract No. N01-AM-7-2213 Starting Date: September 1, 1980
Expiration Date: December 31, 1980

Contractor: University of Geneva, Geneva, Switzerland

Money Allocated: $7,420 Person-years Purchased: 0.3

Conducting Morphological Studies of Diabetes

Under this contract, the detailed morphology of pancreases from Pima autopsy specimens is being studied. The different cell types of the Islets of Langerhans are being quantified using immunofluorescent techniques. Human fibroblasts isolated from diabetic and nondiabetic Pimas will be compared for morphologic differences.

Publications supported by Contract:

1. Malaisse-Lagae, F., Stefan, Y., Cox, J., Perrelet, A., and Orci, L.: Identification of a lobe in the adult human pancreas rich in pancreatic polypeptide. Diabetologia 17:361-365, 1979.

2. Endocrine pancreas: Three-dimensional reconstruction shows two types of islets of Langerhans. Science 206:1323-1325, 1979.

3. Orci, L.: Some recent findings concerning the rodent and human pancreas. In: Current Views of Hypoglycemia and Glucagon: D. Andreani, P.J. Lefebvre and V. Marks (eds): Proc. Serono Symposia Vol. 30, pp. 1-11, Academic Press, 1980.
4. Glicentin: A Precursor of glucagon? Life Sciences 25:287-290, 1979.

5. Krejs, G., Orci, L., Conlon, J.M., et al: Somatostatinoma syndrome. New England Journal of Medicine 301:285-292, 1979.

6. Bordi, C., Ravazzola, M., Baetens, D., et al: A study of glucagonomas by light and electron microscopy and immunofluorescence. Diabetes 28:925-936, 1979.

7. Unger, R.H. and Orci, L.: Alpha cell function in diabetes mellitus. In: Current Views on Hypoglycemia and Glucagon: D. Andreani, P.J. Lefebvre and V. Marks (eds): Proc. Serono Symposia Vol. 30, pp. 93-105, Academic Press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69009-16 EFS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Natural History of Arthritis and Rheumatism on the Gila River Indian Community

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	PH. Bennett	Chief	EFSB, NIAMDD
Other:	J.R. Lisse	Staff Physician	SFSS, NIAMDD
	D.J. Pettitt	Assistant Chief	SFSS, NIAMDD
	W.C. Knowler	Chief	SFSS, NIAMDD
	T.T. Kuberski	Medical Officer	EFSB, NIAMDD

COOPERATING UNITS (if any)

Dean Mann, Immunology Branch, National Cancer Institute

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION
Southwestern Field Studies Section

INSTITUTE AND LOCATION
NIAMDD, NIH, Phoenix, Arizona 85014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.0	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The development and progression of various forms of arthritis is being investigated in an American Indian population. The patterns of joint disease will be examined to determine if distinctive patterns of degenerative joint disease can be recognized, and if so, their evolution and outcome will be followed. In addition, the prognostic significance of various factors related to rheumatoid arthritis and ankylosing spondylitis will be examined.

The study which includes clinical radiographic and serologic studies of joint disease is a part of a prospective study of the Gila River Indian Community in which studies of diabetes mellitus and other diseases are being undertaken.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

1. Determine the progression of clinical, radiological and serological features of joint diseases affecting the Gila River Indians with particular attention to rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and gout.

2. Determine environmental and genetic factors associated with development and progression of arthritis and rheumatism.

3. To compare and contrast the clinical course of rheumatoid arthritis in sero-positive individuals with that of sero-negative arthritics.

4. Determine the predictive and prognostic significance of rheumatoid factor in relation to the clinical course of rheumatoid arthritis.

Methods Employed:

1. The following tests and examinations are repeated on all Indians five years of age and over, living on the Sacaton Service Unit portion of the Gila River Reservation.

   a. Clinical interview
   b. Physical examination
   c. Radiographs of hands and feet. Males over age 17 and females over age 44 will have a pelvis radiograph
   d. Laboratory determinations: rheumatoid factor, uric acid

2. The data from the above examinations and tests as well as demographic, pedigree, environmental information, and data on other conditions and diseases are also collected.

3. HLA and DR typing are performed on subjec-s with rheumatoid arthritis, ankylosing spondylitis and appropriate controls.

Major Findings: In patients with rheumatoid arthritis and age-sex matched controls, titers of Epstein-Barr virus nuclear antigens (EBNA) were not related to the disease. IgA antibody to VCA showed a weak association with the bentonite flocculation titer. In addition the titers of EBNA in the Pima sample as a whole were very high, suggesting chronic antigenic stimulation.

Rheumatoid factor was examined as a risk factor for later development of rheumatoid arthritis. The incidence of rheumatoid arthritis increased from 1.61 cases/1000 person-years in subjects with reumatoid factor titers of 1:4 or less to 6.16 cases/1000 person-years in subjects with rheumatoid factors of at least 1:64. In men at least 25 years old and women at least 45 years old, the age-specific incidence of rheumatoid arthritis was much higher in Pimas than in the Caucasian population of Rochester, Minnesota. These high incidence rates in older Pimas could indicate a cohort effect in the adult Pimas, or that the exposure of Pimas to the environmental risk factor for rheumatoid arthritis occur later in life than in Caucasians. The magnitude of the difference in the older age groups suggests that the Pimas are, however, exposed to more stimuli than Caucasians, or respond differently.

Significance to Biomedical Research and the Program of the Institute: Establishing the environmental risk factors and as well as the host susceptibility to rheumatoid arthritis are important in determining the etiology of this disease. In addition characterizing the risk factors for severe erosive disease can best be done by a longitudinal examination of the radiographs and correlating this data with some of the factors known to be associated with rheumatoid arthritis.

Proposed Course: Further delineation of risk factors for development of rheumatoid arthritis as well as those for development of severe disease will continue. This method will be applied to other forms of arthritis, including neuropathic disease of the foot in diabetics and osteoarthrosis.

Publications:

1. Lisse, J.R., Kuberski, T.T., Bennett, P.H., Knowler, W.C., Gofton, J.P., Calin, A., and Mann, D.L.: High Risk of Sacroiliitis in HLA-B27-Positive Pima Men. Arthritis and Rheumatism (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 69012-03 EFS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Descriptive Epidemiology of Glomerulonephritis and Other Urinary Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Jeffrey A. Cutler Epidemiologist EFS, NIADDK

COOPERATING UNITS (if any)
Missouri Kidney Program, University of Missouri, Columbia, Mo.
Department of Medical Statistics and Epidemiology, Mayo Clinic
Office of Biometry and Field Studies, NINCDS, NIH
Various divisions, National Center of Health Statistics

LAB/BRANCH
Epidemiology and Field Studies Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

INACTIVE

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM70500-03 CMO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on Insulin's Regulation of Glucose Transport and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lester B. Salans	Chief	CMOS, NIADDK
	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
Other:	Paul J. Hissin	Staff Fellow	CMOS, NIADDK
	Eddy Karnieli	Visiting Fellow	CMOS, NIADDK
	Ian A. Simpson	Visiting Associate	CMOS, NIADDK

COOPERATING UNITS (if any) Phoenix Clinical Research Section, NIADDK, NIH, Phoenix, AZ; Section of Biochemistry, Cornell University, Ithaca, NY: Institute of Physiology, University of Aarhus, Aarhus, Denmark; Laboratories of Metabolic Research, University of Geneva, Geneva, Switzerland

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.00	5.00	3.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of insulin's interaction with it specific receptors in the plasma membrane of the rat adipose cell is under investigation through 1) the development of a mathematical model of the complex kinetics of insulin binding and degradation, and 2) an examination of insulin receptor internalization. A further characterization of the insulin-induced translocation of glucose transport systems from a large intracellular pool to the plasma membrane in the rat adipose cell is in progress through an examination of the kinetics of this process, and of the chemical and immunological nature of the rat adipose cell glucose transporter. The role of this translocation process in insulin's stimulatory action on glucose transport in rat diaphragm and the human adipose cell has now been suggested. A possible mechanism of insulin resistant glucose transport in the adipose cell in the aging rat model of obesity, with high fat:low carbohydrate feeding in the rat, and in the streptozotocin-induced diabetic rat model of insulin-dependent diabetes mellitus has been established with the demonstration of a relative depletion of the intracellular pool from which glucose transport systems are translocated to the plasma membrane in response to insulin in all three of these perturbed metabolic states.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM70502-03 CMO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Insulin's Regulation of Glucose Transport

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
	Ian A. Simpson	Visiting Associate	CMOS, NIADDK
Other:	Eddy Karnieli	Visiting Fellow	CMOS, NIADDK
	Paul J. Hissin	Staff Fellow	CMOS, NIADDK
	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any) Phoenix Clinical Research Section, NIADDK, NIH, Phoenix, AZ; Section of Biochemistry, Cornell University, Ithaca, NY; Laboratories of Metabolic Research, University Geneva, Geneva, Switzerland

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.35	2.65	1.70

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A specific D-glucose-inhibitable cytochalasin B binding assay for quantitating the number of glucose transport systems in subcellular membrane fractions has previously been used to demonstrate that insulin stimulates glucose transport in the isolated rat adipose cell primarily through the translocation of glucose transport systems from a large intracellular pool to the plasma membrane. This intracellular pool is associated with the cell's low-density microsomal membranes. This assay has now been used to demonstrate that this translocation process in the rat adipose cell is multi-stepped, extremely rapid, completely reversible, and insulin concentration-dependent. In addition, insulin appears to stimulate glucose transport in rat diaphragm and the isolated human adipose cell through this same translocation mechanism. Furthermore, the rat adipose cell's intracellular glucose transport systems appear to be 1)localized in either a unique subfraction of the Golgi apparatus or a distinct membrane species, 2) immunologically similar to the human erythrocyte glucose transporter with a MW of roughly 45,000 daltons, and 3) chemically processed during their cycling to the plasma membrane in response to insulin.

PHS-6040
(Rev. 2-81)

incubation of these two membrane fractions prepared from basal cells (absence of insulin) gives a distribution of available binding sites which parallels that of the plasma membrane marker enzyme, 5'-nucleotidase. On exposure of cells to insulin (0-3.7 nM), there is a rapid and insulin concentration-dependent appearance of insulin binding sites in the low-density microsomal fraction. However, these binding sites are only detectable when measured in the presence of detergent. These results suggest that insulin receptors in the isolated rat adipose cell are internalized in response to insulin from the plasma membrane into an intracellular pool associated with the same membrane fraction from which glucose transport systems are translocated to the plasma membrane.

Publications: None

In the second series of experiments, the internalization of ^{125}I-insulin and insulin receptors was then examined by incubating samples of adipose cells from the stock cell suspension for varying periods of time up to 60 minutes at 37°C in the absence or presence of 10 mM KCN or at 15°C in the presence of tracer ^{125}I-insulin and 0.17 μM (2.5 U/ml) native insulin. The incubated cells were then homogenized, mitochondrial, plasma membrane, and high- and low-density microsomal membrane fractions were prepared by differential ultracentrifugation, and the distribution of ^{125}I-insulin among these fractions was examined by incubating samples of each for 18 hours at 4°C in the absence or presence of 17 μM (2.5 U/ml) native insulin.

Results and Discussion: Tracer ^{125}I-insulin rapidly associates with the isolated rat adipose cell in a time- and temperature-dependent manner. At lower incubation temperatures, tracer binding ultimately attains a steady state level. With increasing incubation temperature, however, insulin degradation increases such that at 37°C tracer binding reaches an early maximal level, then declines. When high concentrations of native insulin are added to the cells during the association period, further tracer binding is prevented and that tracer already bound to the cells rapidly dissociates. The rate of dissociation appears to depend upon the concentration of native insulin added, the duration of the association period, and the incubation temperature. A one binding site mathematical model of this complex process cannot satisfactorily account for the observed results although the degradation portion of this model does appear to explain the decrease in tracer association with longer incubation periods at higher temperatures. A two site mathematical model of insulin binding has, however, now been formulated which can explain both insulin association and dissociation at a given insulin concentration. This model permits estimation of the rate constants for each site with reasonable certainty, as well as the proportion of the total number of sites in each class. Nevertheless, a simple two site model cannot account for the relationship between insulin binding and insulin concentration. Two variations in the model are, therefore, under consideration: one in which the proportion of sites in each class varies with hormone concentration, and one in which the rate constants vary.

The complexity of ^{125}I-insulin's interaction with the isolated rat adipose cell may, in part, be related to its internalization. Such an internalization of insulin has previously been observed by other investigators. Internalization of the insulin receptor itself, however, has not been established. Since insulin stimulates glucose transport in this cell primarily through a rapid, reversible, and concentration-dependent translocation of glucose transport systems to the plasma membrane from a large intracellular pool associated with low-density microsomal membranes, the distribution of insulin receptors between the plasma and low-density microsomal membrane fractions has been examined in collaboration with Dr. Ole Sonne. Firstly, incubation of adipose cells with 1 nM ^{125}I-insulin causes an accumulation of ^{125}I-insulin in the low-density microsomes. This accumulation of ^{125}I-insulin is inhibited by low temperature or 10 mM KCN. Secondly, Objectives: Insulin's hypoglycemic effect in man and experimental animal *in vivo* has long been recognized, as has been insulin's ability to stimulate the uptake and metabolism of glucose by such peripheral tissues as muscle and adipose tissue. Indeed, insulin's effects on peripheral tissue glucose metabolism *in vivo* are closely paralleled by similar effects on the metabolism of glucose by isolated muscle fibers and adipose cells *in vitro*. While adipose tissue and its constituent adipose cells probably do not represent a primary site of glucose utilization in overall glucose homeostasis in the intact organism, except perhaps in the young and rapidly growing animal, its availability and the homogeneity of the cells prepared therefrom render this tissue an ideal model system with which to examine the mechanism(s) of insulin action at the cellular and subcellular levels. The overall objective of the present investigation is to elucidate the mechanism through which insulin interacts with the rat, and ultimately human, adipose cell, the initial event in insulin's regulatory action on glucose metabolism. This mechanism comprises a complex interaction of insulin with its specific receptors in the adipose cell's plasma membrane, internalization and/or degradation of the bound insulin, and initiation of the signaling process which eventually culminates in a rapid stimulation of glucose transport into the adipose cell, enhancement of the activity of a variety of specific pathways for glucose carbon metabolism in the adipose cell, and inhibition of the stimulatory effect of a variety of agents on the adipose cell's lipolytic activity. The specific objectives for the current year have been 1) continue the development of a mathematical model of the kinetics of ^{125}I-insulin association with, dissociation from, and degradation by, the isolated rat adipose cell, and 2) initiate a study of the specific role of ^{125}I-insulin and insulin receptor internalization in this complex interaction of insulin with the isolated rat adipose cell.

Methods: In all experiments, isolated adipose cells were prepared by incubating the whole epididymal fat pads of male Sprague Dawley rats weighing 150-200 g with collagenase, separating the liberated adipose cells from the stromal-vascular elements by thorough washing, and resuspending the washed cells as a stock cell suspension. Adipose cell size and concentration in the stock cell suspension were determined using an osmic acid fixation, Coulter Electronic Counter method.

In the first series of experiments, the kinetics of ^{125}I-insulin association was then examined by incubating samples of adipose cells from the stock cell suspension for varying periods of time up to 300 minutes at temperatures varying from 15-37°C in the presence of tracer ^{125}I-insulin and 0-17 μM (2.5 U/ml) native insulin. The dissociation of bound ^{125}I-insulin was examined by adding additional native insulin at varying concentrations up to 17 μM (2.5 U/ml) and continuing the incubation for varying additional periods of time up to 300 minutes. The incubated cells were assayed for bound ^{125}I-insulin and the incubation medium for degraded ^{125}I-insulin. The results of these experiments were analyzed using the SAAM27 compartmental modeling program implemented on an IBM 1108 computer at the National Bureau of Standards.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70501-03 CMO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Insulin-Cell Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ian A. Simpson	Visiting Associate	CMOS, NIADDK
OTHER:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK

COOPERATING UNITS (if any)
Institute of Physiology, University of Aarhus, Aarhus, Denmark

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.20	0.90	0.30

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Insulin's interaction with its specific receptors in the plasma membrane of the adipose cell, the initial event in the regulation of glucose transport and metabolism by insulin, is complex. The mechanism of this interaction is under investigation through 1) the development of a mathematical model of ^{125}I-insulin binding and degradation, and 2) an examination of the role of ^{125}I-insulin and insulin receptor internalization in the overall binding process. To date, a one binding site model of insulin's interaction with the adipose cell has been rejected although some of the kinetics of insulin association can be successfully explained by hormone degradation. While a two binding site model can explain the kinetics of both association and dissociation at a given insulin concentration, the parameters of each site or the proportion of sites with constant parameters must vary with insulin concentration. A rapid and insulin concentrationdependent internalization of insulin receptors in the adipose cell has now been demonstrated. Internalized receptors appear in the same low-density microsomal membrane fraction with which the cell's intracellular pool of glucose transport is associated and from which glucose transport systems are translocated to the plasma membrane in response to insulin.

High fat:low carbohydrate relative to high carbohydrate:low fat feeding is associated with a marked reduction in insulin's ability to stimulate glucose transport in the isolated rat adipose cell. Since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of high fat:low carbohydrate feeding on this pool and the translocation process in the isolated rat adipose cell have been examined. With high fat:low carbohydrate feeding, insulin's ability to stimulate 3-O-methylglucose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells is decreased by 55% and 41%, respectively. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from cells of the high fat:low carbohydrate-fed rats is decreased by 47%. Thus, the reduced glucose transport response of the rat adipose cell to insulin with high fat:low carbohydrate feeding appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

The streptozotcoin-induced diabetic rat model of insulin-dependent diabetes mellitus is associated with a marked reduction in insulin's ability to stimulate glucose transport in the isolated adipose cell. Since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of streptozotocin-induced diabetes on this pool and the translocation process in the isolated adipose cell have been examined. With the induction of diabetes, insulin's ability to stimulate 3-O-methylglucose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells is decreased by 55% and 40%, respectively. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from cells of the diabetic animals is decreased by 45%. Thus, the reduced glucose transport response of the adipose cell to insulin in the streptozotocin-induced diabetic rat appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

Publications: None

binding process. To date, a one binding site model of insulin's interaction with the adipose cell has been rejected although some of the kinetics of insulin association can be successfully explained by hormone degradation. While a two binding site model can explain the kinetics of both association and dissociation at a given insulin concentration, the parameters of each site or the proportion of sites with constant parameters must vary with insulin concentration. A rapid and insulin concentrationdependent internalization of insulin receptors in the adipose cell has now been demonstrated. Internalized receptors appear in the same low-density microsomal membrane fraction with which the cell's intracellular pool of glucose transport is associated and from which glucose transport systems are translocated to the plasma membrane in response to insulin.

A specific D-glucose-inhibitable cytochalasin B binding assay for quantitating the number of glucose transport systems in subcellular membrane fractions has previously been used to demonstrate that insulin stimulates glucose transport in the isolated rat adipose cell primarily through the translocation of glucose transport systems from a large intracellular pool to the plasma membrane. This intracellular pool is associated with the cell's low-density microsomal membranes. This assay has now been used to demonstrate that this translocation process in the rat adipose cell is multi-stepped, extremely rapid, completely reversible, and insulin concentration-dependent. In addition, insulin appears to stimulate glucose transport in rat diaphragm and the isolated human adipose cell through this same translocation mechanism. Furthermore, the rat adipose cell's intracellular glucose transport systems appear to be 1) localized in either a unique subfraction of the Golgi apparatus or a distinct membrane species, 2) immunologically similar to the human erythrocyte glucose transporter with a MW of roughly 45,000 daltons, and 3) chemically processed during their cycling to the plasma membrane in response to insulin.

Increasing adipose cell size in the aging rat model of obesity is accompanied by a marked reduction in insulin's ability to stimulate glucose metabolism. Since a fundamental action of insulin in regulating glucose metabolism is its stimulatory effect on glucose transport and since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of increasing cell size on this pool and the translocation process in the isolated adipose cell have been examined in relation to glucose transport activity in the intact cell. With a 10-fold increase in cell size, insulin's ability to stimulate L-arabinose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells practically disappears. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from the enlarged cells is decreased by 80%. Thus, adipose cellular enlargement is accompanied by a markedly reduced glucose transport response to insulin which appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

Objectives: Insulin's ability to stimulate the uptake and metabolism of glucose by such peripheral tissues as muscle and adipose tissue and to inhibit lipolysis in the latter tissue play a major role in systemic glucose and lipid homeostasis *in vivo* in man and experimental animal. Insulin resistance states, exemplified by obesity and diabetes mellitus, are accompanied, in turn, by perturbed systemic glucose and lipid homeostasis and a reduction in the capacity of the peripheral tissues to respond to insulin. Increasing ratios of dietary carbohydrate to fat, on the other hand, tend to ameliorate these insulin resistant states. The alterations in peripheral tissue function observed *in vivo* in these altered physiological and pathophysiological metabolic states are closely paralleled by similar alterations in adipose and muscle cell function studied *in vitro*. The overall objective of the Cellular Metabolism and Obesity Section is an elucidation of the mechanism of insulin action and its perturbation in obesity and diabetes and with altered dietary carbohydrate and fat content. Over the past several years, this objective has been approached through a detailed examination of the isolated adipose cell as a model system. The Section's specific objectives comprise the five major projects now presently in progress: 1) insulin-cell interactions, 2) insulin's regulation of glucose transport, 3) alterations in insulin's action in obesity, 4) role of dietary carbohydrate and fat in modifying insulin's action, and 5) alterations in insulin's action in insulin-dependent diabetes mellitus.

Progress: The mechanism of insulin's interaction with it specific receptors in the plasma membrane of the rat adipose cell is under investigation through 1) the development of a mathematical model of the complex kinetics of insulin binding and degradation, and 2) an examination of insulin receptor internalization. A further characterization of the insulin-induced translocation of glucose transport systems from a large intracellular pool to the plasma membrane in the rat adipose cell is in progress through an examination of the kinetics of this process, and of the chemical and immunological nature of the rat adipose cell glucose transporter. The role of this translocation process in insulin's stimulatory action on glucose transport in rat diaphragm and the human adipose cell has now been suggested. A possible mechanism of insulin resistant glucose transport in the adipose cell in the aging rat model of obesity, with high fat:low carbohydrate feeding in the rat, and in the streptozotocin-induced diabetic rat model of insulin-dependent diabetes mellitus has been established with the demonstration of a relative depletion of the intracellular pool from which glucose transport systems are translocated to the plasma membrane in response to insulin in all three of these perturbed perturbed metabolic states.

Insulin's interaction with its specific receptors in the plasma membrane of the adipose cell, the initial event in the regulation of glucose transport and metabolism by insulin, is complex. The mechanism of this interaction is under investigation through 1) the development of a mathematical model of ^{125}I-insulin binding and degradation, and 2) an examination of the role of ^{125}I-insulin and insulin receptor internalization in the overall

Objectives: Insulin's ability to stimulate the uptake and metabolism of glucose by such peripheral tissues as muscle and adipose tissue has long been recognized as one of the primary mechanisms through which this hormone contributes to systemic glucose homeostasis in vivo in both man and experimental animal. More recently, glucose transport activity has been implicated as the rate limiting step for glucose metabolism in the adipose, and probably muscle, cell under most physiological conditions. The entry of glucose into the adipose cell is mediated by a highly specific facilitated diffusion process. Insulin's stimulatory action on glucose uptake and metabolism in this cell occurs, in turn, primarily through an increase in the maximum transport velocity, either by increasing the mobility of preexisting transport systems which are functional in the absence of insulin, by increasing the total number of transport systems which continue to function at a constant mobility, or by some combination of the two. The overall objective of the present investigation is to characterize the adipose cell's glucose transporter proteins and elucidate the process through which their activities are modulated by insulin. Previously, a specific D-glucose-inhibitable equlibrium ^{3}H-cytochalasin B binding assay has been developed for quantitating the number of glucose transport systems in the purified plasma membranes of isolated rat adipose cells and used to demonstrate that insulin stimulates glucose transport in the rat adipose cell primarily by increasing the number of functional glucose transport systems. More recently, this same assay has been used to identify a large intracellular pool of glucose transport systems associated with the rat adipose cell's low-density microsomal membranes and to demonstrate that insulin increases the number of glucose transport systems in the cell's plasma membrane through their translocation from this intracellular pool. The specific objectives for the current year have been to 1) examine the kinetics of this translocation process in the rat adipose cell, 2) determine if a similar process occurs in rat muscle and the human adipose cell, and 3) initiate an investigation of the mechanism through which this translocation occurs.

Methods: Isolated rat adipose cells were prepared by incubating the whole epididymal fat pads of male Sprague-Dawley rats weighing 150-200 g with collagenase, separating the liberated adipose cells from the stromal-vascular elements by thorough washing, and resuspending the washed cells as a stock cell suspension. Samples of adipose cells from the stock cell suspension were then incubated for varying times at 37°C in the absence or presence of 0.7 or 7.0 nM (100 or 1000 μU/ml) insulin, washed in a tris, EDTA, sucrose buffer, and then homogenized. Various membrane fractions were collected using well established differential ultracentrifugation and sucrose gradient techniques, washed, and resuspended in homogenization buffer.

Isolated human adipose cells were prepared in a similar fashion from samples of subcutaneous and omental fat obtained from random patients undergoing elective surgery. Samples of human adipose cells were then incubated and various membrane fractions collected as just described for rat adipose cells.

Rat diaphragms were removed from 125-150 g Wistar rats, washed, and incubated for 30 minutes at 37°C in the absence or presence of 280 nM (10 mU/ml) insulin. The incubated diaphragms were then washed in a tris, EDTA, sucrose buffer, homogenized, and various membrane fractions collected using a high salt, differential ultracentrifugation method developed specifically for muscle.

Equilibrium cytochalasin B binding was measured at 4°C by incubating membranes in the presence of increasing concentrations of ^{3}H-cytochalasin B up to 2000 nM and unlabelled cytochalasin B at 0 or 20 µM. The membranes were separated from the incubation medium by centrifugation, and the membrane pellet and incubation medium were analyzed for bound and free ^{3}H-cytochalasin B, respectively. Free ^{3}H-cytochalasin B trapped in the membrane pellet was determined using U-^{14}C-sucrose.

Results and Discussion: Incubation of intact isolated rat adipose cells with 0.7 nM (100 µU/ml) insulin at 37°C rapidly increases the number of D-glucose-inhibitable cytochalasin B-binding sites in the plasma membrane fraction from 10 to 55 pmol and simultaneously decreases that in the low-density microsomal membrane fraction from 95 to 34 pmol, each with a half-time of approximately 2.5 min. Further, when maximally insulin-stimulated intact cells are exposed to a 300-fold excess of anti-insulin antibody, both effects of insulin are simultaneously reversed by 95% over a 30 min period with half-times of approximately 9 min. The concentrations of insulin producing half-maximal and maximal effects are approximately 0.11 nM (15 µU/ml) and 0.35 nM (50 µU/ml), respectively. The patterns of these reciprocal effects of insulin in time, during reversal, and with increasing hormone concentration are highly correlated ($r = 0.93$). At steady state, the magnitude of insulin's stimulatory effect on the number of D-glucose-inhibitable cytochalasin B-binding sites in the plasma membrane fraction closely correlates with the magnitude of insulin's stimulatory action on 3-O-methylglucose transport in the intact cell. However, during the onset of insulin's action, the appearance of cytochalasin B binding sites in the plasma membrane fraction preceeds the rise in 3-O-methylglucose transport in the intact cell (half-time of 4.0 min) by approximately 1.5 min. No such lag is observed during the reversal of insulin's action. These results directly demonstrate that insulin increases the number of glucose transport systems in the plasma membrane of the isolated rat adipose cell through a rapid and reversible translocation of glucose transport systems from a specific intracellular membrane pool. While this translocation of glucose transport systems to the plasma membrane fully accounts for the magnitude of insulin's stimulatory action on glucose transport activity in the intact cell, it appears to represent only one step in a multi-step process.

In collaboration with Dr. Lawrence J. Wardzala, cytochalasin B binding has been used to quantitate the number of glucose transport systems in plasma and microsomal membranes prepared from intact rat diaphragm. In a series of three experiments, plasma membranes prepared from diaphragms which have not been incubated with insulin, bind approximately 16 pmol of cytochalasin B/mg of membrane protein to the D-glucose-inhibitable binding site. If 280 nM (40,000 μU/ml) insulin is present during the incubation, cytochalasin B binding to the plasma membranes is increased approximately 2-fold. Membranes in the microsomal fraction prepared from diaphragms which have been incubated for 30 min in the absence of insulin contain 21 pmol of D-glucose-inhibitable cytochalasin B binding sites/mg of membrane protein. However, in the presence of insulin during the incubation period, the number of these sites in the microsomal fraction is decreased to 12 pmol/mg of membrane protein. These results suggest that insulin stimulates glucose transport in the isolated rat diaphragm primarily through a translocation of glucose transport systems from an intracellular membrane pool to the plasma membrane similar to that observed in rat adipose cells, and suggest that this mechanism of insulin-stimulated glucose transport activity may be general to other cell types.

In a preliminary series of experiments in collaboration with Dr. James E. Foley, plasma, high-density microsomal, and low-density microsomal membrane fractions have been prepared from isolated human adipose cells following their incubation in the absence or presence of 7.0 nM (1000 μUml) insulin. In the basal state, the plasma and low-density microsomal membranes from these human cells contain approximately 2 and 19 pmol of D-glucose-inhibitable cytochalasin B binding sites/mg of membrane protein, respectively. In the maximally insulin-stimulated state, on the other hand, the number of such sites in the plasma membrane fraction is increased roughly 2.5-fold while that in the low-density microsomal membrane fraction is decreased by roughly 50%. Insulin enhances 3-O-methylglucose transport in the intact cell 3-5-fold. Thus, insulin also appears to stimulate glucose transport in the human adipose cell through the translocation of glucose transport systems from an intracellular pool to the plasma membrane. Quantitatively, the human adipose cell is similar to the enlarged adipose cell of the aged, obese rat.

In collaboration with Drs. Peter C. Hinkle, Thomas J. Wheeler, and David C. Sogin, the cytochalasin B binding assay and a rabbit IgG prepared against the purified human erythrocyte glucose transporter have been used to further characterize the intracellular pool of glucose transport systems and this translocation process in isolated rat adipose cells. When membrane fractions are prepared from cells incubated without insulin, roughly 90% of the cells' total glucose transport systems measured by cytochalasin B binding are found in the low-density microsomal fraction. However, while galactosyltransferase activity, a Golgi enzyme marker, is enriched in the low-density microsomal membrane fraction, its distribution over all fractions does not parallel that of the glucose transport systems. In response to insulin, roughly 50% of the intracellular glucose transport systems are translocated to the plasma membrane fraction without a corresponding redistribution of enzyme marker activities. In the plasma membrane fraction prepared from basal and insulin-stimulated cells, the Kd of the glucose transport systems for cytochalasin B is 98±10 nM. In contrast, the Kd of intracellular glucose transport systems is 141±15 nM in the basal state and decreases to that observed in the plasma membrane fraction in response to insulin. The subcellular distribution of glucose transport systems and their insulin-stimulated redistribution have been confirmed qualitatively using an electrophoretic gel transfer technique, a rabbit IgG prepared against the purified human erythrocyte glucose transporter, and ^{125}I-protein A and autoradiography. A major band of 45,000 MW is revealed in both the plasma and low-density microsomal membrane fractions, its intensity varying according to the concentration of glucose transport systems as determined by cytochalasin B binding. Thus, the rat adpose cell's intracellular glucose transport systems appear to be 1) localized in either a unique subfraction of the Golgi apparatus or a distinct membrane species, 2) immunologically similar to the human erythrocyte glucose transporter, and 3) chemically processed during their cycling to the plasma membrane in response to insulin.

Publications:

Karnieli, E., M.J. Zarnowski, P.J. Hissin, I.A. Simpson, L.B. Salans, and S.W. Cushman. Insulin-stimulated translocation of glucose transport systems in the isolated rat adipose cell: time course, reversal, insulin concentration-dependency and relationship to glucose transport activity. J. Biol. Chem. 256:4772-4777, 1981.

Wardzala, L.J., and B. Jeanrenaud. Potential mechanism of insulin action on glucose transport in the isolated rat diaphragm: apparent translocation of intracellular transport systems to the plasma membrane. J. Biol. Chem. in press, 1981.

Cushman, S.W., L.J. Wardzala, E. Karnieli, P.J. Hissin, I.A. Simpson, and L.B. Salans. Regulation of glucose transport by insulin: reversible translocation of intracellular glucose transport sytems to the plasma membrane. Interntl. J. Obesity, in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70503-03 CMO

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Alterations in Insulin's Action in Obesity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. Hissin	Staff Fellow	CMOS, NIADDK
Other:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.65	0.35	0.30

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Increasing adipose cell size in the aging rat model of obesity is accompanied by a marked reduction in insulin's ability to stimulate glucose metabolism. Since a fundamental action of insulin in regulating glucose metabolism is its stimulatory effect on glucose transport and since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of increasing cell size on this pool and the translocation process in the isolated adipose cell have been examined in relation to glucose transport activity in the intact cell. With a 10-fold increase in cell size, insulin's ability to stimulate L-arabinose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells practically disappears. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from the enlarged cells is decreased by 80%. Thus, adipose cellular enlargement is accompanied by a markedly reduced glucose transport response to insulin which appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

PHS-6040
(Rev. 2-81)

Objectives: Increasing adipose cell size in the aging male Sprague-Dawley rat model of obesity is accompanied by alterations in adipose cellular metabolic function in vitro which parallel those observed in human adipose cells in vitro in both spontaneous and experimental obesity, as well as the alterations in systemic glucose and lipid metabolism accompanying obesity in man and the aging rat. The overall objective of the present investigation is to describe these alterations in rat adipose cell function in detail and to elucidate the mechanisms responsible for these alterations. Since previous investigations have demonstrated that insulin appears to stimulate glucose transport activity in the rat adipose cell by triggering a rapid and reversible translocation of glucose transport systems from an intracellular pool to the plasma membrane, the specific objective for the current year has been to examine the subcellular distribution of glucose transport systems in the enlarged adipose cells of aged, obese rats in relation to glucose transport activity in the intact cell.

Methods: Small and large isolated adipose cells were prepared by incubating the whole epididymal fat pads of young, lean and old, obese male Sprague-Dawley rats with collagenase, separating the liberated adipose cells from the stromal-vascular elements by thorough washing, and resuspending the washed cells as a stock cell suspension. Adipose cell size and concentration in the stock cell suspension were determined using an osmic acid fixation, Coulter Electronic Counter method.

The number of glucose transport systems was then determined by measuring D-glucose-inhibitable equilibrium ^{3}H-cytochalasin B binding to the plasma, high-density microsomal, and low-density microsomal membrane fractions prepared from samples of the isolated adipose cells which were first incubated for 30 minutes in the absence or presence of 7 nM (1000 μU/ml) insulin. Scatchard analysis of 6 point cytochalasin B binding isotherms in the absence or presence of 500 mM D-glucose permits quantitation of the number of specific cytochalasin B binding sites in the class of sites which has been identified as the glucose transport system. Differences in the number of these sites between the small and large cells were also compared to differences in glucose transport activity in the same preparations of cells by estimating the latter with a ^{14}C-L-arabinose uptake technique.

Results and Discussion: Basal glucose transport activity increases from 0.3 to 1.4 fmol/cell/min with a 10-fold increase in cell size, but remains constant per unit cellular surface area and is accompanied by a constant 5 pmol of glucose transport systems/mg of membrane protein in the plasma membrane fraction. Maximally insulin-stimulated glucose transport activity, on the other hand, remains constant at 2.3 fmol/cell/min with increasing cell size, but markedly decreases per unit cellular surface area and is accompanied by a decrease from 30 pmol of glucose transport systems/mg of plasma membrane protein to the basal level. These diminished effects of insulin on glucose transport activity and the number of glucose transport systems in the plasma membrane fraction in enlarged cells are paralleled by an 80% decrease in the basal number of glucose transport systems/mg of membrane protein in the low-density microsomal membrane fraction, the source of those glucose transport systems appearing in the plasma membrane in response to insulin. These alterations occur without significant changes in the membrane species comprising those fractions examined here, as reflected in the distributions of marker enzyme activities. The effects of cell size on the metabolism of a low concentration of 1-^{14}C-glucose (0.56 mM) directly parallel those on glucose transport activity and the concentration of glucose transport systems in the plasma membrane fraction, and are not associated with significant alterations in the cell's sensitivity to insulin.

Although the "insulin resistance" of the enlarged adipose cell in the aging rat model of obesity is well established, the mechanisms responsible are not fully understood. The present investigations suggest that 1) a primary locus of this resistance to insulin resides at the glucose transport level, and 2) insulin resistance at the glucose transport level can be explained by a marked decrease in the number of glucose transport systems appearing in the plasma membrane in response to insulin as the consequence of a relative depletion of these glucose transport systems in the cell's intracellular pool.

Publications:

Cushman, S.W., D. Noda, and L.B. Salans. Adipose cell size-function relationships: insulin binding and degradation. Amer. J. Physiol. 240:E166-E174, 1981.

Cushman, S.W., P.J. Hissin, L.J. Wardzala, J.E. Foley, I.A. Simpson, E. Karnieli, and L.B. Salans. Mechanism of insulin resistance in the adipose cell in the aging rat model of obesity. Biochem. Soc. Trans., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70504-03 CMO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Alterations in Adipose Cell Function in the Genetically Obese Zucker Fatty Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS: 0.00	PROFESSIONAL: 0.00	OTHER: 0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Temporarily inactive.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70505-03 CMO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of Dietary Carbohydrate and Fat in Modifying Insulin's Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. Hissin	Staff Fellow	CMOS, NIADDK
OTHER:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.45	OTHER: 0.30

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

High fat:low carbohydrate relative to high carbohydrate:low fat feeding is associated with a marked reduction in insulin's ability to stimulate glucose transport in the isolated rat adipose cell. Since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of high fat:low carbohydrate feeding on this pool and the translocation process in the isolated rat adipose cell have been examined. With high fat:low carbohydrate feeding, insulin's ability to stimulate 3-O-methylglucose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells is decreased by 55% and 41%, respectively. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from cells of the high fat:low carbohydrate-fed rats is decreased by 47%. Thus, the reduced glucose transport response of the rat adipose cell to insulin with high fat:low carbohydrate feeding appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

Objectives: Variations in the ratio of dietary carbohydrate to fat in young, lean Sprague-Dawley rats are accompanied by alterations in adipose cellular metabolic function *in vitro* which parallel those observed in human adipose cells *in vitro*, as well as the alterations in systemic glucose and lipid metabolism *in vivo* accompanying such manipulations of dietary composition in both man and rat. In addition, many of the alterations, at both the systemic and cellular levels, produced by decreasing ratios of dietary carbohydrate to fat parallel those observed with increasing adipose cell size in both spontaneous and experimental obesity in man and in the aging male Sprague-Dawley rat model of obesity. The overall objective of the present investigation is to describe these dietary-induced alterations in rat adipose cell function in detail, to relate these alterations to those observed in the aging Sprague-Dawley rat, and to elucidate the mechanisms through which the multiple variables represented by these diverse models interact in regulating net adipose cellular metabolic activity. Since previous investigations have demonstrated that insulin appears to stimulate glucose transport activity in the rat adipose cell by triggering a rapid and reversible translocation of glucose transport systems from an intracellular pool to the plasma membrane, the specific objective for the current year has been to examine the subcellular distribution of glucose transport systems in the adipose cells of young, lean rats raised on either a high carbohydrate:low fat or a high fat:low carbohydrate diet. The glucose transport response to insulin is markedly diminished in the latter, relative to the former.

Methods: Weanling rats were raised for three weeks on one of two experimental diets of equicaloric density containing either a high (71%:9%) or a low (30%:50%) ratio (by calories) of carbohydrate to fat. The protein content of the diets was maintained at a constant 20%. Isolated adipose cells were then prepared from these rats by incubating the whole epididymal fat pads with collagenase, separating the liberated adipose cells from the stromal-vascular elements by thorough washing, and resuspending the washed cells as a stock cell suspension. Adipose cell size and concentration in the stock cell suspension were determined using an osmic acid fixation, Coulter Electronic Counter method.

The number of glucose transport systems was then determined by measuring D-glucose-inhibitable equilibrium ^{3}H-cytochalasin B binding to the plasma, high-density microsomal, and low-density microsomal membrane fractions prepared from samples of the isolated adipose cells which were first incubated for 15 minutes in the absence or presence of 7 nM (1000 μU/ml) insulin. Scatchard analysis of 6 point cytochalasin B binding isotherms in the absence or presence of 500 mM D-glucose permits quantitation of the number of specific cytochalasin B binding sites in a class of sites which has been identified as the glucose transport system. Alterations in the number of these sites with altered dietary composition were also compared to alterations in glucose transport activity in the same preparations of cells by estimating the latter with a ^{14}C-3-O-methylglucose uptake technique in additional samples of cells from the same stock cell suspensions.

Results and Discussion: Basal glucose transport activity is decreased from 0.37 to 0.19 fmol/cell/min in cells from rats raised on the high fat:low carbohydrate diet compared to the high carbohydrate:low fat diet, but is accompanied by a constant 10 pmol of glucose transport systems/mg of membrane protein in the plasma membrane fraction. Maximally insulin-stimulated glucose transport activity is decreased from 4.63 to 2.10 fmol/cell/min, and is accompaniedd by a decrease from 44 to 26 pmol of glucose transport systems/mg of plasma membrane protein. The diminished effects of insulin on glucose transport activity in the intact cell and the concentration of glucose transport systems in the plasma membrane fraction are paralleled by a 47% decrease in the basal number of glucose transport systems/mg of membrane protein in the low-density microsomal membrane fraction.

The results of these investigations demonstrate that the isolated rat adipose cell's resistance to insulin at the glucose transport level with high fat:low carbohydrate feeding is accompanied by 1) a markedly diminished stimulatory effect of insulin on the concentration of glucose transport systems in the plasma membrane fraction, closely paralleling the markedly decreased stimulatory effect of insulin on 3-O-methylglucose transport in the intact cell, and 2) a marked reduction in the basal concentration of glucose transport systems in the low-density microsomal membrane fraction, the cell's intracellular pool from which glucose transport systems are translocated to the plasma membrane. These alerations occur without significant changes in the membrane species comprising those fractions examined here, as reflected in the distribution of marker enzyme activities. Thus, the "insulin resistant" glucose transport of the rat adipose cell with high fat:low carbohydrate feeding can be explained by a decrease in the number of glucose transport systems appearing in the plasma membrane in response to insulin as the consequence of a depletion of these glucose transport systems in the cell's intracellular pool.

Publication:

Salans, L.B., J.E. Foley, L.J. Wardzala, and S.W. Cushman. Effects of dietary composition on glucose metabolism in rat adipose cells. Amer. J. Physiol. 240:E175-E183, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM70506-01 CMO

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Alterations in Insulin's Action in Insulin-Dependent Diabetes Mellitus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eddy Karnieli	Visiting Fellow	CMOS, NIADDK
OTHER:	Samuel W. Cushman	Associate Chief	CMOS, NIADDK
	Lester B. Salans	Chief	CMOS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.05	0.65	0.40

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The streptozotcoin-induced diabetic rat model of insulin-dependent diabetes mellitus is associated with a marked reduction in insulin's ability to stimulate glucose transport in the isolated adipose cell. Since insulin appears to enhance glucose transport by triggering a rapid and reversible translocation of glucose transport systems from a large intracellular pool to the plasma membrane, the effects of streptozotocin-induced diabetes on this pool and the translocation process in the isolated adipose cell have been examined. With the induction of diabetes, insulin's ability to stimulate 3-O-methylglucose transport in intact cells and to increase the concentration of glucose transport systems in the plasma membrane fraction prepared from these cells is decreased by 55% and 40%, respectively. At the same time, the basal concentration of glucose transport systems in the low-density microsomal membrane fraction prepared from cells of the diabetic animals is decreased by 45%. Thus, the reduced glucose transport response of the adipose cell to insulin in the streptozotocin-induced diabetic rat appears to be the consequence of a relative depletion of glucose transport systems in the intracellular pool.

PHS-6040
(Rev. 2-81)

Objectives: The adipose cell in the streptozotocin-induced diabetic rat model of insulin-dependent diabetes mellitus is characterized by alterations in adipose cellular metabolic function in vitro which parallel those observed in human adipose cells in vitro in this pathophysiological state, as well as the alterations in systemic glucose and lipid metabolism accompanying insulin-dependent diabetes mellitus in man and the streptozotocin-induced diabetic rat. The overall objective of the present investigation is to describe these alterations in rat adipose cell function in detail and to elucidate the mechanisms responsible for these alterations. Since previous investigations have demonstrated that insulin appears to stimulate glucose transport activity in the rat adipose cell by triggering a rapid and reversible translocation of glucose transport systems from an intracellular pool to the plasma membrane, the specific objective for the current year has been to examine the subcellular distribution of glucose transport systems in the adipose cells of streptozotocin-induced diabetic rats where the glucose transport response to insulin is markedly diminished.

Methods: Streptozotocin-diabetic and control rats were obtained by intravenously injecting 150-200 g male Sprague-Dawley rats with either a freshly prepared solution of streptozotocin in 0.1 mM citrate buffer, 65 mg/kg body weight, or buffer alone, respectively, seven days prior to sacrifice. Isolated adipose cells were then prepared from these rats by incubating the whole epididymal fat pads with collagenase, separating the liberated adipose cells from the stromal-vascular elements by thorough washing, and resuspending the washed cells as a stock cell suspension. Adipose cell size and concentration in the stock cell suspension were determined using an osmic acid fixation, Coulter Electronic Counter method.

The number of glucose transport systems was then determined by measuring D-glucose-inhibitable equilibrium ^{3}H-cytochalasin B binding to the plasma, high-density microsomal, and low-density microsomal membrane fractions of the isolated adipose cells which were first incubated for 15 minutes in the absence or presence of 7 nM (1000 μU/ml) insulin. Scatchard analysis of 6 point cytochalasin B binding isotherms in the absence or presence of 500 mM D-glucose permits quantitation of the number of specific cytochalasin B binding sites in a class of sites which has been identified as the glucose transport system. Alterations in the number of these sites with streptozotocin-induced diabetes were also compared to alterations in glucose transport activity in the same preparations of cells by estimating the latter with a ^{14}C-3-O-methylglucose uptake technique in additional samples of cells from the same stock cell suspensions.

Results and Discussion: Basal glucose transport activity decreases from 0.19 to 0.12 fmol/cell/min with the induction of diabetes, but remains constant per unit cellular surface area and is accompanied by a constant 6 pmol of glucose transport systems/mg of membrane protein in the plasma membrane fraction. Maximally insulin-stimulated glucose transport activity decreases from 3.16 to 1.05 fmol/cell/min and from 0.26 to 0.12 amol/μm^2/min, and is accompanied by a decrease from 25 to 15 pmol of glucose transport systems/mg of plasma membrane protein. These diminished effects of insulin on glucose transport activity and the concentration of glucose transport systems in the plasma membrane fraction are paralleled by a 45% decrease in the basal number of glucose transport systems/mg of membrane protein in the low-density microsomal membrane fraction.

The results of these investigations demonstrate that the isolated adipose cell's resistance to insulin at the glucose transport level in the streptozotocin-induced diabetic rat is accompanied by 1) a markedly diminished stimulatory effect of insulin on the concentration of glucose transport systems in the plamsa membrane fraction, closely paralleling the markedly decreased stimulatory effect of insulin on 3-O-methylglucose transport in the intact cell expressed per unit cellular surface area, and 2) a marked reduction in the basal concentration of glucose transport systems in the low-density microsomal membrane fraction, the cell's intracellular pool from which glucose transport systems are translocated to the plasma membrane. These alterations occur without significant changes in the membrane speices comprising those fractions examined here, as reflected in the distributions of marker enzyme activities. Thus, the "insulin resistant" glucose transport of the adipose cell in this animal model of insuin-dependent diabetes mellitus can be explained by a decrease in the number of glucose transport systems appearing in the plasma membrane in response to insulin as the consequence of a depletion of these glucose transport systems in the cell's intracellular pool.

Publications:

Karnieli, E., P.J. Hissin, I.A. Simpson, L.B. Salans, and S.W. Cushman. A possible mechanism of insulin resistance in the rat adipose cell in streptozotocin-induced diabetes mellitus: depeletion of intracellular glucose transport systems. *J. Clin. Invest.*, in press, 1981.

ANNUAL REPORT

OFFICE OF SCIENTIFIC AND TECHNICAL REPORTS

The Office of Scientific and Technical Reports (OSTR) prepares, produces and disseminates information about the varied research and program activities of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK). This information takes many forms: brochures, pamphlets, fact sheets, background papers, announcements, news releases, reports to the Congress, responses to public and congressional inquiries, exhibits at scientific meetings, and importantly, contact with print and broadcast media representatives.

Formerly known as the National Institute of Arthritis, Metabolism, and Digestive Diseases, the Institute was renamed in Title II of the Health Programs Extension Act of 1980 (P.L. 96-538). The announcement of the name change was made following publication of a notice in the Federal Register on June 23, 1981. The new name more accurately reflects the major extramural research programs of the Institute: Arthritis, Musculoskeletal and Skin Diseases Program; Diabetes, Endocrine and Metabolic Diseases Program; Digestive Diseases and Nutrition Program; and Kidney, Urologic and Blood Diseases Program.

Information responsibilities are divided along these lines, with an information specialist designated to cover the activities of each research area. A major vacancy in arthritis information was filled in December 1980 giving coverage to this major area of Institute responsibility. Reporting on the Intramural Research Program, with the exception of the Diabetes Branch, has been assigned to a writer-editor, who is also correspondent for The NIH Record. Coverage of research on systemic lupus erythematosus was emphasized by assigning an editorial assistant to that subject as part of the special STRIDE training program. The exhibits program, undergoing substantial re-evaluation in preparation for 1982, is managed by an editorial assistant.

Organizational changes in the Institute in the past year resulted in expansion of OSTR to include professional and technical information responsibilities in addition to public information activities. Two members of the former Scientific Communications Office were reassigned to OSTR: a technical information specialist, who is also the Institute MEDLINE Coordinator, and an editorial assistant for technical publications. Support staff include one secretary and one public inquiries clerk. OSTR also provided training assignments to two interns in support of public information goals at NIH.

PUBLICATIONS PROGRAM

During fiscal year 1981, OSTR continued its "series" approach to the publications program. A unified graphic design was used in each of four series of publications: brochures, pamphlets, fact sheets and reports. Approximately 70,000 publications were distributed to the public from July 1980 to July 1981.

Reports on arthritis included the Fourth Annual Report of the Director on the Arthritis Program and NIADDK 1981: Arthritis and Related Rheumatic Disorders. A new brochure on arthritis has been completed and is currently in press. A pamphlet entitled What You Should Know about Vitiligo was produced and distributed to the lay public.

A special report, 1981 Diabetes Mellitus: Trans-NIH Research, was published to highlight research advances over the past year. A Report of NIH Diabetes Mellitus Coordinating Committee was produced by NIADDK to highlight programmatic initiatives in diabetes. A fact sheet, Diabetes: An Overview, summarized research advances and legislation that have led to more effective treatment and control of this major health problem.

New publications on cystic fibrosis included NIADDK 1981: Cystic Fibrosis, a report on research advances in the field, and Cystic Fibrosis: The Puzzle and the Promise, a pamphlet describing the nature and treatment of the disorder.

In the digestive diseases area, What is Pancreatitis?, a fact sheet, was produced. A report on research advances, NIADDK 1981: Digestive Diseases, was published for distribution at scientific meetings. The pamphlet Peptic Ulcer won an Award of Achievement from the Society for Technical Communication in January 1981.

Research results were published in the report NIADDK 1981: Kidney and Urinary Tract Diseases and Treatment. This report and a new pamphlet, Understanding Urinary Tract Infection, were among the publications distributed at major medical and scientific meetings. A fact sheet, Continuous Ambulatory Peritoneal Dialysis, was also produced and later reprinted by the NIH News and Features.

In the past year, OSTR coordinated its information activities with the congressionally mandated Arthritis Information Clearinghouse, National Diabetes Information Clearinghouse, and National Digestive Diseases Education and Information Clearinghouse, each of which serves as a distribution arm for publications in their respective research areas.

CONGRESSIONAL INQUIRIES

The Office of Scientific and Technical Reports handles constituent correspondence referred from the Congress and the White House. Because of active public interest in a wide-ranging number of subject areas within its research purview, NIADDK receives a large volume of these requests.

During the period July 1980 through May 1981, OSTR prepared 234 written responses to inquiries from Senators, Congressmen, the Secretary, of DHHS, and the White House, an increase of 78 over the previous year. The OSTR also received 530 telephone inquiries from congressional staff members.

Most congressional inquiries concern the funding of individual grants and specific research programs; lobbying efforts of numerous voluntary health agencies; proposed "cures" for various ailments; the federal funding status of a particular research area; requests from constituents for financial assistance; and requests for information from ill constituents, their families, and friends.

PUBLIC INQUIRIES

A significant portion of the U.S. public is directly concerned with the disease areas under investigation by NIADDK. An estimated 60 million people in the United States are victims of arthritis, diabetes and digestive diseases alone. During the period July 1980 through May 1981, OSTR received approximately 4,598 telephone inquiries from patients, their relatives and friends, students, allied health professionals, physicians and scientists. In addition, 320 letters were written in response to public inquiries, exclusive of requests for publications or congressional inquiries, an increase of 30 percent more than in 1979-80.

Requests for information dealt with questions about controversial forms of therapy, how to become a patient in an NIADDK research study, financial aid, physician referral, accepted diagnosis and treatment procedures, proposed "cures" for certain diseases, and NIADDK research grant and contract programs.

PRESS ACTIVITIES

Contact with representatives of print media is an integral part of OSTR's public information program. In support of the established policy to publicize NIADDK's programs and activities, OSTR reaches a variety of media outlets through personal contact and distribution of news releases and announcements.

Two articles on osteoporosis, "Avoiding Brittle Bones," _Better Homes and Gardens_, August 1980, and "Your Bones--Stop Starving Them," _Self_, May 1981, featured remarks by Dr. G. Donald Whedon.

Lynn Lamberg of _Better Homes and Gardens_ won the Arthritis Foundation's 1980 press award for an article on arthritis prepared with assistance from OSTR staff.

An article on apheresis, an experimental technique used to treat rheumatoid arthritis and other disorders, was the cover story of the June 8, 1981, issue of _Medical World News_. Arthritis and Rheumatism Branch Chief Dr. John Decker was quoted in the article, entitled "New Blood-Washing Therapy."

The _Washington Post_ column "Health Talk" featured an interview about systemic lupus erythematosus with Dr. Lawrence E. Shulman. The story was arranged by OSTR staff member Mrs. Barbara Weldon.

A major article on Kawasaki's disease, referencing NIADDK grantees, appeared in the June 6, 1981 issue of the _Washington Post_.

Dr. Laurence H. Miller was the subject of an interview in U.S. News and World Report on June 22, 1981, entitled "How to Save your Skin While Enjoying the Summer Sun."

In the diabetes research area, the successful transplantation of insulin producing cells from rats to mice by NIADDK grantee Dr. Paul E. Lacy was the subject of an article in The New York Times on July 7, 1980. On the same day, a similar story heavily based on a news release, prepared by OSTR staff member Mrs. Patricia Sheridan, appeared in the Washington Post.

The first artificial pancreas implanted in a human diabetic, performed by NIADDK grantees, was featured in the Washington STAR on November 8, 1980.

A major article on diabetes, referencing NIADDK research and grantees, appeared in the May 1981 issue of Better Homes and Gardens. The article generated approximately 1,500 letters to the National Diabetes Information Clearinghouse within two weeks of publication.

The Washington Post and the Washington Star ran stories on a new test developed by NIADDK grantees to identify parents carrying genes for cystic fibrosis on January 1, 1981.

The New York Times ran an article on May 15, 1981, on NIADDK research on prophylactic prenatal vitamin supplementation to prevent a rare genetic defect of the newborn. The production of human growth hormone using gene-splicing techniques was the subject of a feature article, which referenced NIADDK grantees, in the Science Times section of The New York Times, January 6, 1981

"What You Should Know About Ulcers," a by-lined article written by Dr. Harold P. Roth and OSTR staff member Mr. Irving Shapiro, appeared in the June 1980 issue of Occupational Health and Safety. A feature article entitled "Ulcers: Modern Treatment for an Ancient Ailment," referencing NIADDK, appeared in the September 1980 issue of Changing Times.

Endoscopy guidelines resulting from an NIADDK-sponsored Consensus Development Conference were the topic of a feature article in September 15, 1980 issue of Medical World News. The results of an NIADDK-supported study on Crohn's disease were reported in the NIH Alumni Association Newsletter. NIADDK obesity research was quoted in a July 1980 article in the Washington Post concerning weight loss camps for children.

Dr. Harold P. Roth was quoted in an article on diet for ulcer patients that appeared in the April 1981 issue of Better Homes and Gardens. He was also quoted in articles on ulcers that appeared in several daily papers, including the Newark Star Ledger on August 16, 1980.

Results of NIADDK's study of the drug CDCA for the treatment of cholesterol gallstones were reported in the Journal of the American Medical Association on June 19, 1981, and in U.S. News and World Report and Medical World News on June 22, 1981.

Two articles, "Insulin and Human Growth Hormone: Triumphs of Genetic Engineering" and "Prolonged Survival of Rat Islet Cells in Diabetic Mice Offers Hope," appeared in the prestigious "From the NIH" column of the Journal of the American Medical Association.

OSTR prepared a number of news releases during the past year, including announcements on the successful transplantation of insulin-producing cells from rats to mice, the Consensus Development Conference on endoscopy, the NIADDK-sponsored symposium on genetics research, a new treatment for a rare skin disease, the appointment of three new members to the NIH Arthritis, Diabetes, and Digestive and Kidney Diseases Advisory Council, the results of a 10-year study on the dissolution of gallstones, and the Institute's name change.

RADIO AND TELEVISION

A number of NIADDK scientists were featured this year on radio and television broadcasts, both locally and nationally.

Dr. Lawrence Shulman, associate director of the Arthritis, Musculoskeletal and Skin Diseases Program, was interviewed about systemic lupus erythematosus (SLE) on the nationally-distributed Newsweek TV in November 1980 and on the local Channel 9 Panorama program in January 1981. He was also featured on a taped interview for WGMS and other national radio stations on the subject of SLE.

Dr. Harold Roth, associate director of the Digestive Diseases and Nutrition Program, discussed Crohn's disease in an interview for WGMS radio in September 1980. Dr. John Decker discussed rheumatoid arthritis on WGMS radio in April 1981.

Dr. James Balow talked about lupus nephritis on Channel 9's Panorama program in conjunction with a Medicine for the Layman lecture on September 30, 1980. Dr. Phillip Gordon gave a Medicine for the Layman lecture on diabetes on November 18, 1980.

EXHIBITS

OSTR has responsibility for coordinating the exhibit program for NIADDK, designed primarily for a scientific audience. Six major exhibits were viewed at 14 medical and scientific meetings throughout the United States by more than 55,000 physicians, scientists, and allied health professionals.

Institute exhibits on nephrology, human growth hormone, cystic fibrosis, digestive diseases, diabetes, and general research were displayed at meetings such as those of the Federation of American Societies for Experimental Biology, the American Gastroenterological Association, the American Diabetes Association, the American Public Health Association, the College of American Pathologists, and the Minority Biomedical Symposium. Appropriate publications were distributed by OSTR staff members who accompanied exhibits.

During the past year, the exhibits program has phased out "crated" exhibits and includes only displays that are easily updated and portable. In addition to designing new portable displays on nephrology and digestive diseases, individual panels were updated and replaced for the diabetes and NIADDK general research exhibits.

In a cooperative effort with the Indian Health Service, NIADDK has arranged for the loan of a diabetes exhibit on the PIMA Indians to the PHS Indian Health Hospital in Albuquerque, New Mexico on a long term basis. The exhibit will eventually be returned to the Institute's Southwestern Field Studies Section in Phoenix, Arizona for permanent display.

The exhibits program continues to be a valuable method of reaching a primary audience of NIADDK, the scientific and medical community. For example, 15 digestive diseases publications produced with NIADDK support were distributed at two national gastroenterological meetings in the past year. Thousands of extramural publications, program announcements, and other Institute materials are distributed directly to relevant publics at meetings each year.

THE NIH RECORD

A primary method of communication is through The NIH Record, which is distributed to NIH employees, wire services, local news media, science writers and editors, and scientists in the United States and abroad. Because this publication reaches many of NIADDK's relevant publics, a writer-editor is assigned as Record correspondent to provide greater visibility for NIADDK's programs. As a result of this emphasis, the number of items about NIADDK's activities and personnel increased from 16 in 1978-79 to 40 in 1979-80 to 60 in 1980-81.

NIADDK's 30th anniversary received extensive Record coverage. The focal point of the celebration was an all-day public symposium on "DNA, The Cell Nucleus, and Genetic Disease," held at NIH on October 15. Stories announced the symposium, described the symposium agenda, and later announced the availability of tapes of the proceedings. NIADDK also published a four-page feature on highlights of the Institute's current research programs. This special Record issue included stories on intramural investigations in seven laboratories and branches and on research supported by the extramural program in each of the four major disease areas.

NIADDK news releases, described earlier, are published by The NIH Record. In addition, during the past year 60 articles were written for the Record on subjects such as scientific and performance awards, retirements, biomedical research, and conferences and seminars of interest to the NIH community.

RESEARCH REPORTS

Research reports are brief summaries about significant findings reported in the literature by NIADDK scientists and grantees. Prepared by OSTR, these reports reflect the scope of the Institute's research programs, and are used primarily as a basis for special reports to Congress and related reports to DHHS and NIH.

The following examples are representative of the 57 research reports prepared during the past year: 1) improved motor nerve conduction in diabetics treated with portable insulin infusion pumps; 2) cyclophosphamide heals vascular lesions and lowers levels of circulating immune complexes in rheumatoid arthritis; 3) HLA antigens and the inheritance of regional enteritis (Crohn's disease); 4) increased numbers of immunoglobulin-secreting cells in the blood of patients with active systemic lupus erythematosus (SLE); 5) estrogen therapy improves calcium absorption in postmenopausal women; 6) the aminopyrine breath test predicts recovery of liver function from alcohol-induced injury; 7) combined plasmapheresis and immunosuppression promotes recovery from rapidly progressive glomerulonephritis; 8) strict metabolic control of pregnant diabetic mothers prevents excessive insulin secretion by their fetuses; 9) inherited early-onset diabetes in purebred dogs; 10) viruses decrease the number of insulin receptors on cultured human cells.

SPECIAL PROJECTS

OSTR organized activities surrounding the celebration of NIADDK's 30th anniversary on October 15, 1980. Over 500 scientists from NIH, local universities and laboratories attended a full-day symposium at Masur Auditorium, entitled "DNA, the Cell Nucleus and Genetic Disease," sponsored by NIADDK's intramural research program. The symposium was followed by a dinner at the National Naval Medical Center, Bethesda, Maryland, where Dr. Donald Seldin, Chairman, Department of Internal Medicine, University of Texas, Southwestern Medical School, spoke on "NIH and Medicine, a Symbiotic Relationship."

In preparing for the anniversary activities, OSTR coordinated the design and production of a variety of artwork, designed to be visually similar in color and layout, and using a special symbol designating the Institute. OSTR staff wrote and distributed a news release, a series of four NIH Record articles, a special four-page Record spread about the Institute, and an article for the NIH Alumni Association Newsletter (described previously). Information folders were distributed at the symposium, which featured talks by genetics experts, including two Nobel laureates, all of whom are present or former NIADDK employees.

NIH Library, Building 10
National Institutes of Health
Bethesda, Md. 20205

NIADDK sponsored a Consensus Development Conference on the use of endoscopy in upper gastrointestinal bleeding in August 1980. OSTR worked closely with the Office of Medical Applications of Research in preparing and disseminating information materials for the digestive diseases community and the news media.

NIH LIBRARY
4 0140 6925

*U.S. GOVERNMENT PRINTING OFFICE: 1981-0-341-132/3677

LIBRARY
NIH
Amazing Research.
Amazing Help.
http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

DATE DUE

GAYLORD
PRINTED IN U.S.A.

NIH LIBRARY
3 1496 00188 9024